# THE EFFECT OF DIET UPON THE BLOOD THIONEINE OF THE ALBINO RAT [1]

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TWO FIGURES

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While assaying grain samples for the new plant toxicant described by Franke ('34), chemical studies on blood from rats on a variety of dietary regimes were made. In this paper there will be reported the findings for thioneine and uric acid. Glutathione was determined on a few samples of blood to determine whether or not the thioneine variations correlated with glutathione concentrations.

## EXPERIMENTAL

The rats were all of Wistar Institute origin and, with the exceptions of the rats on the potato diet, all were in good physical condition at the time of autopsy. The majority of the animals had served as controls in various experiments and had been kept in individual cages. The rats on the potato diet showed poor growth and certain abnormalities which have been described by Kon ('31).

*Diets.* The barley, corn, wheat, oats, and potato diets had the composition shown in table 1.

[1] Published with the permission of the director of the South Dakota Agricultural Experiment Station as communication no. 4 from the Department of Experiment Station Chemistry.

TABLE 1

| | CORN DIET | WHEAT DIET | BARLEY DIET | OATS DIET | POTATO[1] DIET |
|---|---|---|---|---|---|
| Ground grain | 82.0 | 82.0 | 82.0 | 82.0 | 82.0 |
| Casein | 10.0 | 10.0 | 10.0 | 15.0 | 10.0 |
| McCollum's salt mixture no. 185 | 1.0 | 1.0 | 1.0 | 1.0 | 4.0 |
| Cod liver oil | 2.0 | 2.0 | 2.0 | 1.0 | 2.0 |
| Northwestern dried yeast | 2.0 | 2.0 | 2.0 | 1.0 | 2.0 |
| Lard | 3.0 | 3.0 | 3.0 | ... | ... |

[1] The potatoes used in this diet were sliced, dried, and ground to a fine powder.

The casein-sucrose low-thioneine diet had the following composition:

| | *Per cent* | | *Per cent* |
|---|---|---|---|
| Sucrose | 45 | Butter | 10 |
| Commercial casein | 20 | McCollum's salt | 3 |
| Northwestern dried yeast | 10 | Cod liver oil | 2 |
| Corn starch | 10 | | |

This diet produces excellent growth.

The stock diet consisted of:

| | *Per cent* | | *Per cent* |
|---|---|---|---|
| Wheat | 25.0 | Alfalfa meal | 5.0 |
| Corn | 25.0 | Ground peas | 5.0 |
| Rolled oats | 20.0 | Cod liver oil | 3.0 |
| Fish meal | 10.0 | NaCl | 1.0 |
| Wheat germ | 5.0 | $CaCO_3$ | 1.0 |

## ANALYTICAL METHODS

Blood was obtained from the abdominal aorta by the method of Swanson and Smith ('32). Two mg. of $K_2C_2O_4$ per cubic centimeter of blood were used to prevent clotting. The tungstomolybdic acid method (Benedict, '31) of preparing protein-free filtrates has been used throughout this study. The determination of thioneine and uric acid is essentially a combination of Benedict and Behre's procedure ('31) with the reagents recently described by Folin ('30).[2]

Folin's reagents have been previously used by Salt ('31) for the determination of thioneine. Salt also used a uric acid

[2] This investigation was completed prior to Folin's later article ('33) which does not, however, affect the validity of the results presented here.

standard for his comparisons. Folin's uric acid reagent is preferred, because it is relatively free from phenol reagent. Furthermore, the use of the cyanide-urea reagent, as described below, eliminates turbidities and the solutions may be compared when cold. As for color values, Folin's uric acid reagent gives colors which are practically identical to the color produced by Benedict's reagent with equal amounts of pure solutions of glutathione or uric acid. Benedict's reagent gives more color with thioneine than does the Folin reagent, but it was thought advisable to use the same reagent for both determinations. Pyrex test tubes 150 × 16 mm. without lip (Board of Health tubes) have been used. These tubes fit the centrifuge cups ordinarily used for 15 cc. conical centrifuge tubes.

*Method for determining uric acid.* To 10 cc. of protein-free filtrate in a test tube add 2.5 cc. of acid lithium chloride (Benedict and Behre, '31). Mix well, and add 2.5 cc. of silver nitrate solution (Benedict and Behre, '31). Shake well, stopper, and centrifuge thoroughly. Carefully pour off top liquid into a clean test tube, and invert the tube with the residue on a pad of filter paper for a few minutes. This residue will be used for the thioneine determination. Pipette 5 cc. of the thioneine-free filtrate into a clean test tube, add 5 cc. of the cyanide-urea (with excess calcium) (Folin, '30) followed by 1 cc. of uric acid reagent (Folin, '30), noting the time. Both reagents should be added from burettes. Invert twice, and stopper with cork. At the end of exactly 4 mintues, immerse in boiling water for exactly 2 minutes. Remove to a rapidly circulating cold water bath for 4 or 5 minutes, centrifuge thoroughly for 1 or 2 minutes, and compare with a suitable standard, which is set at 20 mm. in the left cup of the colorimeter; $20/R \times 0.004 \times$ cc. dilute standard $\times 300 =$ mg. uric acid per 100 cc. The accurate timing during the color development is no hardship once a schedule has been worked out. In this laboratory, cyanide-urea is added to all the tubes, after which the color reagent is added at 10-second intervals to twelve tubes. The color reagent is added to a second group

of twelve tubes while the first group is being cooled. Twenty-four tubes may be run in two groups of twelve each, with but four standards in the series. Turbidities never occur.

*Method of determining thioneine (in terms of uric acid).* Dissolve the silver chloride residue from the indirect uric acid determination (see above) in 5 cc. of cyanide-urea, add 5 cc. of water, followed by 1 cc. of the uric acid reagent and proceed exactly as in the determination of uric acid above; $20/R \times 0.004 \times$ cc. dilute standard $\times 100 =$ UA-mg. of thioneine (thioneine in terms of mg. uric acid) per 100 cc. (whole blood). All thioneine values are reported as UA-mg. of thioneine.

*Standards for thioneine and uric acid.* The stock standard is prepared according to Folin ('30). For the determinations, four standards are prepared, using 0.5 cc., 1 cc., 2 cc., and 3 cc. of the dilute standard and water sufficient to make 5 cc. The standards are run parallel to the blood samples and the four described suffice for both the thioneine and the uric acid determinations.

*Method for determining glutathione.* The method of Benedict and Gottschall ('33) was used,[3] with the exception of the uric acid reagent for the color development, which was that of Folin ('30). The color produced by Folin's uric acid reagent with glutathione in the conditions of this determination are virtually identical with the colors produced by Benedict's reagent, and the former has the advantage of not giving tests for phenols. Volumes of filtrate and reagents were doubled throughout to facilitate color comparison. The authors are aware of the fact that the method has not been previously used on rat blood. Data presented in this paper show quite conclusively that the determination is not affected by thioneine variations in excess of 1000 per cent.

## DISCUSSION OF RESULTS

The results of the thioneine analyses on blood from 134 rats on various diets are shown in figure 1. The levels as observed according to diet were as follows: wheat, $0.43 \pm 0.18$ UA-mg.;

[3] Glutathione from Eastman Kodak Company was used for standards.

corn, 0.93 ± 0.22 UA-mg.; two samples of oats had levels of 1.22 and 0.59 UA-mg., respectively; stock diet yielded an average of 1.17 ± 0.20 UA-mg. A careful study of the data failed to reveal significant differences due to sex or age.

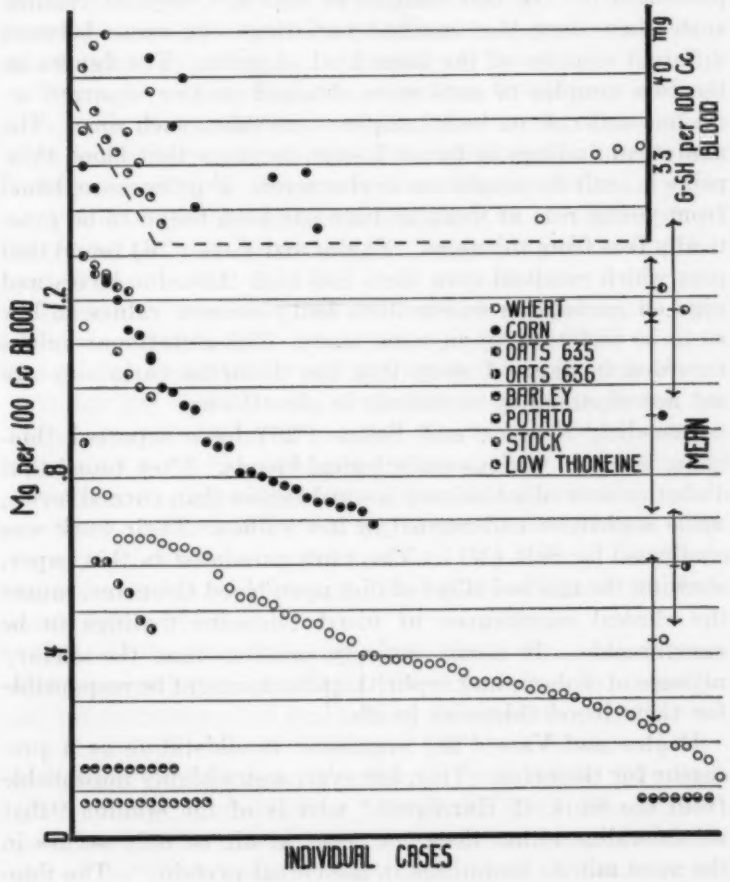


Fig. 1 Results of consecutive blood thioneine determinations on 134 white rats on eight different diets, with mean values and standard deviations for each diet. Glutathione values on twenty-four consecutive cases are given directly above corresponding thioneine values. Thioneine values are expressed in UA-mg. (thioneine in terms of uric acid) and plotted in order of decreasing values.

Figure 1 shows that among the common cereal grains, barley is extremely low in the precursor for thioneine, while corn is rich in the precursor. The wheat mean indicates that wheat is also relatively low in the precursor. The figures presented for the two samples of oats are very interesting, since they show that marked variations can occur between different samples of the same kind of grain. The figures on the two samples of oats were obtained on two separate occasions and rats on both samples were taken each time. The analytical findings in figure 1 seem to prove that blood thioneine is entirely exogenous in character. Furthermore, blood from young rats at weaning time has been found to be practically free from thioneine. Eagles and Vars ('26) found that pigs which received corn diets had high thioneine levels and pigs on garbage or casein diets had thioneine values so low as to be undetectable in some cases. The glutathione values recorded in figure 1 show that the thioneine variations are not accompanied by variations in glutathione.

Benedict, Newton, and Behre ('26) have reported thioneine levels in various pathological bloods. They found that diabetics showed a tendency toward higher than normal levels, while nephritics had normal or low values. Their work was confirmed by Salt ('31). The work presented in this paper, showing the marked effect of diet upon blood thioneine, causes the clinical significance of blood thioneine findings to be questionable. It seems entirely possible that the dietary regimes of diabetic and nephritic patients might be responsible for their blood thioneine levels.

Eagles and Vars ('28) suggested thiolhistidine as a precursor for thioneine. This, however, seems highly improbable from the work of Harington,[4] who is of the opinion "that thiolhistidine either does not occur at all, or only occurs in the most minute quantities in the cereal protein." The thioneine levels observed in the rats on the low-thioneine diet are interesting, because they seem to rule out a number of possible precursors. Casein is comparatively rich in methio-

[4] Personal communication from C. R. Harington.

nine (Pirie, '32) and yeast has been shown to contain considerable glutathione (Tunnicliffe, '25). Since the low-thioneine diet contains 20 and 10 per cent, respectively, of casein and yeast, it seems quite likely that neither methionine nor glutathione is the precursor for thioneine. The fact that the diet was entirely adequate indicates that it was at least not deficient in histidine or cystine.

Figure 2 presents the uric acid values which were observed in all the blood samples used in figure 1. A survey of the data has failed to reveal significant differences in uric acid values according to diet, age or sex. The mean for the group of 127 rats (potato rats excluded) has been calculated and found to be 3.38 mg./100 cc. with a standard deviation of $\pm$ 1.55 mg.

There have been very few publications regarding the blood chemistry of the albino rat. Folin and Morris ('13) analyzed the pooled blood of six normal rats and found 2 mg. of uric acid per 100 cc. of blood. Anderson, Honeywell, Santy, and Pedersen ('30) found an average of 1.86 mg. of uric acid per 100 cc. of blood in seven rats. Turner ('20) found from 2.6 to 3.1 mg. of uric acid per 100 cc. in the blood of twelve normal stock rats and from 2.5 to 3.8 mg. in sixteen control rats. With the exception of the report by Folin and Morris, the figures were based upon tungstic acid filtrates, using Benedict's direct method for uric acid.

Up to this time, thioneine estimations involving the uric acid reagent have utilized the silver residue remaining after the indirect removal of uric acid by means of the silver lactate —acid sodium chloride treatment of blood filtrates (Behre and Benedict, '29; Benedict, Newton, and Behre, '26; Rockwood, Turner, and Pfiiffner, '29; Salt, '31). The author's modification for the thioneine determination involves the use of the silver residue which is obtained in Benedict and Behre's ('31) new method for determining uric acid, and leaves little to be desired from the standpoint of simplicity. Behre ('32) has questioned all methods (Benedict, Newton, and Behre, '26; Rockwood, Turner and Pfiffner, '29; Salt,

'31) involving cyanide as the sole alkali. She states that when sodium hydroxide was employed as the alkali, and a minimum of cyanide was used, lower thioneine figures were obtained (Behre and Benedict, '29), but added thioneine was completely recovered.

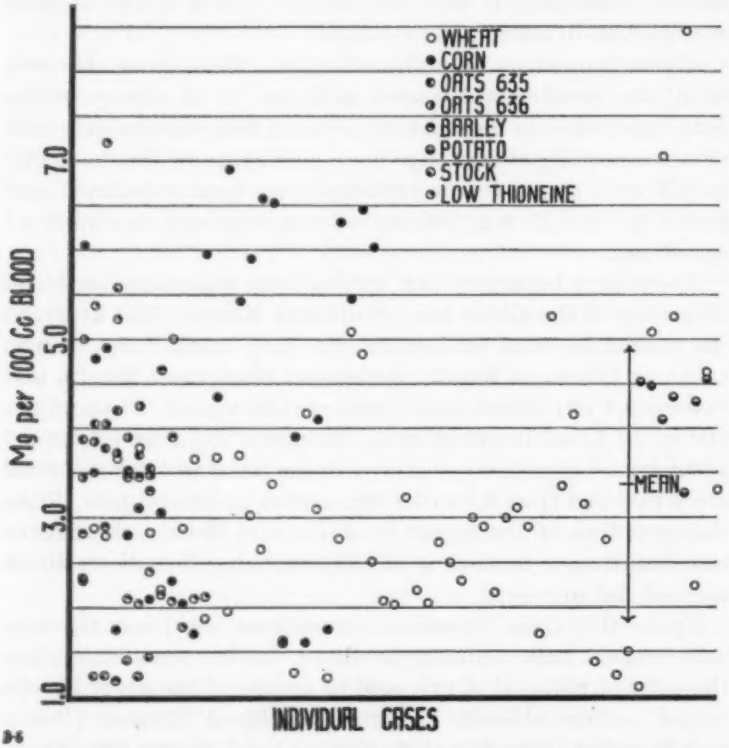


Fig. 2 Results of uric acid determinations on 134 consecutive samples of rat blood in eight different diets, plotted in same order as samples in figure 1. The mean value and standard deviation of the entire group, excluding potato rats, is given.

The authors' method for thioneine is probably in positive error by about 0.075 UA-mg. Experiments in this laboratory have shown that aqueous solutions containing glutathione

equivalent to 21, 35, or 49 mg. per 100 cc. of blood give almost identical colors when analyzed as for thioneine, the color being approximately equal to that given by a solution of uric acid equivalent to 0.075 mg. per 100 cc. The glutathione values for the rats on the low thioneine diet (fig. 1) could therefore account for the thioneine values observed, and the blood of this group of animals may actually have been thioneine-free.

When silver chloride is precipitated in an aqueous solution of glutathione, according to the procedure for thioneine, the glutathione is not quantitatively carried down with the precipitate. Experiments on the inhibitory effect of glutathione upon the uric acid determination, to be published elsewhere, indicate that the indirect uric acid determination may still be affected by glutathione. The authors were unable to make a thorough investigation of the accuracy of the thioneine method by recovery experiments or by determining the effect of possible interfering substances. From a theoretical standpoint, however, the method should be as accurate as that described by Salt ('31) and has the advantages of being much easier to carry out. Thioneine was expressed in terms of uric acid, because it was felt that the extra labor involved would add very little to the significance of the figures presented, which are relatively correct and show clearly the effect of the various cereal grains on blood thioneine. It is hoped that these figures may prove of benefit in the search for a precursor for blood thioneine.

## SUMMARY

1. Blood thioneine is shown to be entirely exogenous and the levels observed in 134 rats on eight different diets are presented.

2. No precursor for thioneine is suggested. The precursor is probably not methionine, glutathione, or thiohistidine.

3. The average of the blood uric acid values in 127 rats is presented.

4. Modified methods for the determination of uric acid and thioneine are presented.

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# LIVER GLYCOGEN FROM DERIVATIVES OF GLUCOSE

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FOUR FIGURES

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Many investigators have studied the ability of the liver to store various foodstuffs in the form of glycogen. The conversion of naturally occurring hexoses (like galactose and fructose) into liver glycogen has been well described (Cori, '26; and Cori and Cori, '27). This type of chemical transformation has even been suggested as a test of liver function (Banks, Sprague and Snell, '33). Nevertheless, the versatility of this chemical synthetic capacity of the hepatic cells has not as yet been completely described in its qualitative aspects.

Drury and Salter ('34) administered a series of glucose derivatives to rabbits whose livers had been removed. They found that substitution of even a single chemical group in the hexose molecule usually rendered the sugar useless for such animals. When given to normal animals (mice), however, many of these carbohydrates were stored as liver glycogen. This paper records liver glycogen formation by six carbohydrates for which data in the extant literature are scanty or lacking. For purposes of comparative standards, this paper also describes the glycogen content of livers in fasting mice when a single feeding of glucose is administered.

The dose of glucose used is varied in successive experiments, so that a considerable range of nutrition is covered. Glycogen storage can be demonstrated in the liver soon after

the sugar is administered; but as fasting continues, the glycogen disappears. The rate of absorption, and the height of liver glycogen depend upon the amount of glucose administered. Four sets of data are presented, representing four levels of carbohydrate storage. It is hoped that the data may serve as standard curves of reference for the study of carbohydrate turnover in mice.

## EXPERIMENTAL PROCEDURE

Young, mature mice of both sexes were used for these experiments. The stock was fed bread, hemp and millet seed, crackers, cabbage, milk, and cod liver oil. Prior to the experiment, they were fasted for 48 hours and then fed through stomach tube a standard amount of sugar in solution. (The selection of standard amounts is described below.) The use of a small glass tube, suitably molded, attached to a tuberculin syringe, permits accurate delivery of liquid. The animals were allowed water ad libitum. They were kept in individual fruit jars in a thermostat controlled animal room at 70° F. At various intervals after feeding, groups of animals were sacrificed.

The animals were stunned, carotid arteries cut, and allowed to bleed for half a minute. The liver was then removed as rapidly as possible and dropped into a tared tube containing 1.5 cc. of 60 per cent warm potassium hydroxide. Each tube was stoppered with a glass bulb, to the undersurface of which a stirring rod was attached. The tissue was speedily disintegrated. The tube was then cooled and weighed again. Ligatures were next placed about the esophagus and rectum and the gastro-intestinal tract removed to a beaker. The contents of the gut were then washed out with several successive portions of hot water, and collected in a volumetric flask. In this manner, unabsorbed sugar could be determined.

## CHEMICAL METHODS

The chemical procedures followed were essentially those described by Cori ('25 a). Glycogen was precipitated after

3 hours' digestion (in aqueous potassium hydroxide at 100° C.) by adding water and alcohol. Two methods were employed in the final estimation of the sugar produced by acid-hydrolysis of the glycogen. The colorimetric method of Folin ('29) proved as accurate as the iodometric titration method of Somogyi, modified from Shaffer-Hartman (Shaffer and Somogyi, '33), but the latter conveniently obviated preliminary determinations of glucose for approximate concentration.

The washings from the gut were deproteinized with colloidal iron as described by Cori ('25 b). The filtrates were then analyzed for reducing substance. Control, fasted animals yielded negligible values by this procedure.

The accuracy of the experimental procedure was controlled by several parallel experimental manipulations. Delivery from the syringe was much more constant than the biological reaction of the animals. The intestines and stomachs of fasted animals yielded negligible reducing values. Livers of 48-hour fasted mice contained (apparently) 0.07 per cent of glycogen which was allowed as a blank. The accuracy of the glycogen determination itself was tested by preparing glycogen from mouse livers by the method of Pflüger, modified by Cori (personal communication), and then analyzing known amounts, both separately and when added to fasting liver tissue. The recovery of added glycogen was $95 \pm 3$ per cent of theoretical.

## INGESTION LEVEL

All the results here reported concern the fate of carbohydrate solutions delivered into the stomach. The maximum amount which can be administered in this fashion depends, first, upon the capacity of the stomach and, secondly, upon the tolerance of the gut to irritating hypertonic solutions. Mice weighing about 25 gm. were selected, and solutions so made up that about $\frac{1}{2}$ cc. of solution was given. The actual amount of sugar to be administered was determined by the weight of each animal. Regurgitation and diarrhea were

carefully looked for, and such animals excluded. Even in the absence of diarrhea, the intestine may be markedly distended by fluid soon after feeding a large amount of sugar.

It was found that the gut tolerates more of glucose than of most other sugars. Accordingly, glucose feeding was conducted at several distinct levels (maximal, two intermediate, and low) so that the effect of other sugars could be compared at similar ingestion levels.

### SPECIAL CARBOHYDRATES STUDIED

Of the special carbohydrate substances used, five were hexose derivatives. These included sorbitol, glucosamine, gluconic acid, and the two glucosides of methyl and ethyl alcohol, respectively. The chemical configuration of these or similar compounds is given by Herring, Irvine and Macleod ('24), as well as by Armstrong ('24). The sorbitol was purchased from Pfanstiehl Company. The gluconate (calcium salt) was generously contributed by Sandoz Chemical Company. The other hexose derivatives were prepared in this laboratory: e.g., $\alpha$-ethyl d-glucoside.

In addition, a glycerol derivative, propylene glycol (propane-diol 1, 2) was also tested. This propylene glycol was obtained from the National Carbon Company through the courtesy of Dr. Reid Hunt.

### EXPERIMENTAL DATA FOR GLUCOSE

Table 1 shows both the change in liver glycogen concentration and in the fraction of the glucose fed which was recovered as glycogen. Two sets of data are given for the maximal dosage curve, one obtained during the winter months and the other in the summer. No significant difference is evident. For the latter curve, pedigreed inbred black mice were employed as well as stock white mice. They showed no obvious difference in behavior.

The maximal level is presented in duplicate. This last level represents the maximum amount of glucose which the gastrointestinal tract would tolerate consistently.

TABLE 1

*Recovery of liver glycogen in mice after feeding glucose in various doses*

| | | TIME AFTER FEEDING | NUMBER OF MICE | | | AVERAGE GLYCOGEN CONTENT OF LIVER | AVERAGE FRACTION OF INGESTED GLUCOSE AS LIVER GLYCOGEN |
|---|---|---|---|---|---|---|---|
| | | | Used | Discarded | Died | | |
| | | *Hours* | | | | *Per cent* | *Per cent* |
| Molecular glucose—equivalent: 0.1 gm. per 100 gm. of mouse | | 4½ | 6 | 1 | | 0.09 | 4.7 |
| | | 6 | 3 | | | 0.12 | 5.2 |
| | | 8 | 6 | | | 0.13 | 6.4 |
| | | 13½ | 8 | 1 | | 0.14 | 6.9 |
| Molecular glucose—equivalent: 0.2 gm. per 100 gm. of mouse | | 4 | 9 | | | 0.85 | 22.3 |
| | | 6½ | 7 | | | 0.75 | 17.8 |
| | | 12½ | 6 | | | 0.48 | 11.7 |
| | | 24½ | 7 | | | 0.08 | 2.3 |
| Molecular glucose—equivalent: 0.5 gm. per 100 gm. of mouse | | 1 | 5 | | | 1.20 | 10.9 |
| | | 2 | 5 | | | 2.00 | 20.3 |
| | | 3 | 4 | 1 | | 2.49 | 22.3 |
| | | 4 | 8 | | | 1.64 | 17.0 |
| | | 6 | 16 | 1 | | 1.11 | 11.8 |
| | | 10 | 12 | 1 | | 0.69 | 6.8 |
| | | 15 | 8 | 2 | | 1.16 | 11.5 |
| | | 20 | 9 | 2 | | 0.51 | 4.6 |
| Molecular glucose—equivalent: 0.7 gm. per 100 gm. of mouse | Summer curve | 4½ | 12 | | | 2.77 | 17.7 |
| | | 4½ | 6 | | | 2.21 | 16.4 |
| | | 4½ | 9 | | | 2.40 | 14.4 |
| | | 6 | 4 | | | 4.39 | 26.5 |
| | | 10 | 5 | | | 2.70 | 19.0 |
| | | 18 | 7 | | | 0.96 | 5.9 |
| | | 24 | 5 | | | 0.29 | 1.5 |
| | Winter curve | ¼ | 2 | | | 0.83 | 7.8 |
| | | ½ | 3 | 1 | | 0.85 | 7.1 |
| | | 1 | 14 | | | 1.17 | 10.8 |
| | | 2 | 10 | | | 1.93 | 14.6 |
| | | 3 | 12 | | | 2.85 | 20.3 |
| | | 4 | 13 | | | 2.49 | 19.1 |
| | | 5 | 11 | 1 | | 3.00 | 24.2 |
| | | 6 | 7 | | | 2.71 | 19.8 |
| | | 7 | 5 | | | 2.42 | 19.9 |
| | | 11 | 4 | | | 2.44 | 18.1 |
| | | 13 | 4 | | | 2.13 | 16.2 |
| | | 15 | 4 | | | 2.03 | 12.4 |
| | | 16 | 4 | | | 1.78 | 11.3 |
| | | 17 | 4 | | | 1.44 | 8.6 |
| | | 20 | 6 | 2 | | 1.30 | 9.7 |
| | | 24 | 7 | 2 | 2 | 0.85 | 4.7 |
| | | 30 | 5 | | | 0.47 | 2.8 |
| | | 40 | 3 | | 3 | 0.88 | 5.5 |
| | | 48 | 4 | 1 | | 0.14 | 0.8 |
| | | Blank | 10 | | | 0.07 | |

The rate of intestinal absorption for the highest level is given in table 2. These data for mice, obtained by a method similar to Cori's ('26) procedure in rats, show a somewhat different trend in relation to time from the results obtained on rats.

Figure 1 shows that the postprandial content of glycogen in the liver becomes progressively higher as increasing

TABLE 2

*Intestinal absorption in mice after feeding glucose and derivatives of glucose*

| | TIME AFTER FEEDING | NUMBER OF MICE | AVERAGE ABSORPTION PERCENTAGE OF CARBOHYDRATE FED |
|---|---|---|---|
| | *Hours* | | *Per cent* |
| Glucose | ¼ | 3 | 24.6 |
| | ½ | 4 | 59.2 |
| | 1 | 6 | 78.8 |
| | 2 | 2 | 91.4 |
| | 3 | 4 | 95.7 |
| | 4 | 5 | 96.5 |
| | 5 | 3 | 96.9 |
| Propylene glycol | ½ | 3 | 77.8 |
| | 1 | 3 | 61.6 |
| | 1½ | 3 | 74.7 |
| | 2 | 6 | 81.8 |
| | 3 | 8 | 84.7 |
| | 5 | 3 | 93.7 |
| | 7 | 6 | 93.6 |
| Glucosamine | 2 | 3 | 45.4 |
| | 5 | 4 | 80.6 |
| Ethyl glucoside | 2 | 3 | 46.1 |
| | 5 | 1 | 58.2 |

amounts of glucose are administered. The limiting value is fixed, not by the liver, but by gastro-intestinal tolerance to a single large feeding. That is, up to the point where diarrhea interferes, liver glycogen concentration is greater when larger amounts of glucose are fed.

By way of contrast, the fraction of fed glucose which the liver stores remains nearly the same despite wide variation in the actual amount fed. This is not true when a very small

amount of sugar is fed; but applies over a considerable range of dosage. Figure 2 shows that the total glycogen stored in the previously fasted liver is roughly proportional to the amount of glucose fed, provided that the dose of sugar is a significant portion of the maximal dose which is tolerated.

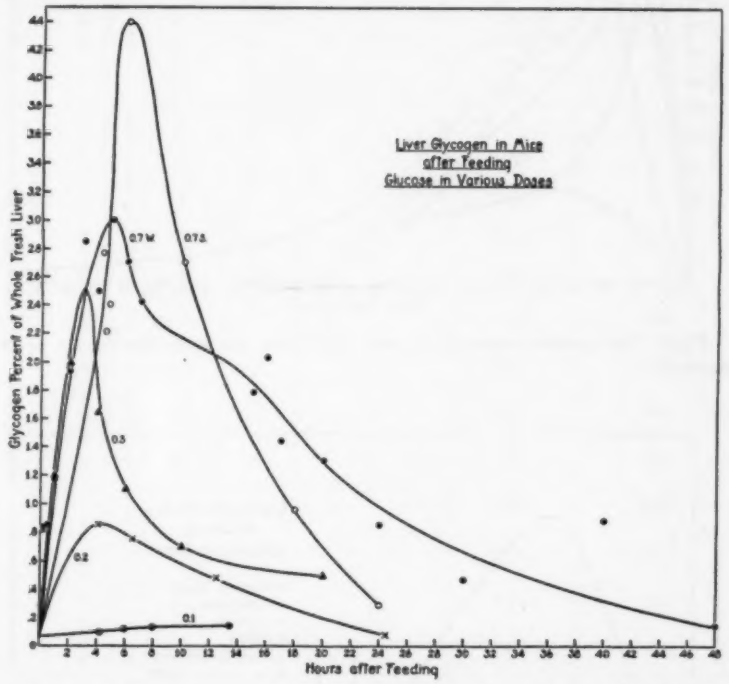


Fig. 1 The figures attached to each curve indicate the grams of glucose fed per 100 gm. of mouse. For 0.7 gm., both a winter and a summer curve are given.

## EXPERIMENTAL DATA FOR SPECIAL CARBOHYDRATES

The results of the liver glycogen determinations after feeding derivatives of glucose are presented in table 3. Results are calculated in terms of total glycogen recovered, as well as in terms of the per cent of glycogen in the liver. The figures are plotted in figure 3 for comparison with the (similarly fed) molecular equivalents of glucose, given in figures 1 and 2.

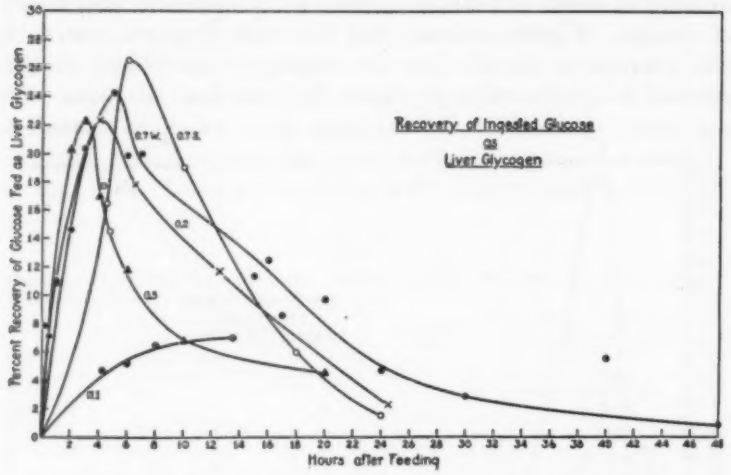


Fig. 2 The symbols attached to each curve have the same significance as in figure 1.

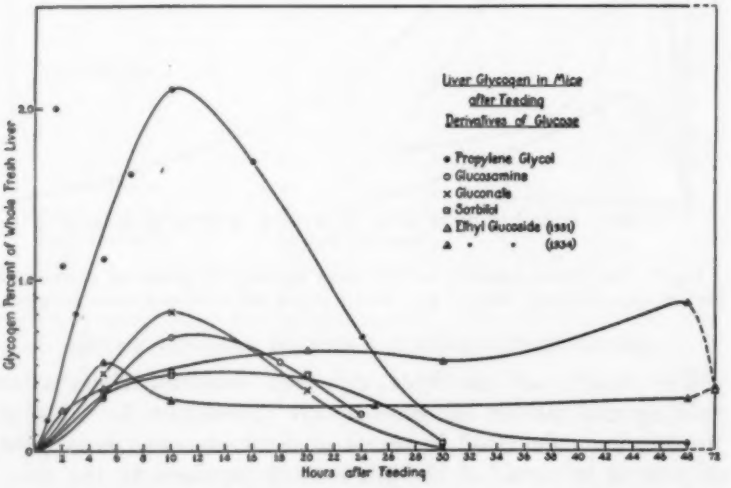


Fig. 3 The amounts of each carbohydrate fed are given in table 3. For ethyl glucoside, data are plotted as obtained in two separate years (1931 and 1934).

TABLE 3

*Recovery of liver glycogen in mice after feeding derivatives of glucose*

| | | TIME AFTER FEEDING | NUMBER OF MICE | | | AVERAGE GLYCOGEN CONTENT OF LIVER | AVERAGE FRACTION OF INGESTED CARBOHYDRATES |
|---|---|---|---|---|---|---|---|
| | | | Used | Discarded | Died | | |
| | | *Hours* | | | | *Per cent* | *Per cent* |
| *Propylene glycol feeding* | Molecular glucose—equivalent: 0.75 gm. per 100 gm. of mouse | 1 | 3 | | | 0.18 | 1.2 |
| | | 1½ | 3 | | | 2.00 | 13.1 |
| | | 2 | 9 | | | 1.08 | 7.8 |
| | | 3 | 10 | | | 1.80 | 5.4 |
| | | 5 | 8 | | | 1.12 | 9.6 |
| | | 7 | 7 | | | 1.62 | 10.9 |
| | | 10 | 4 | | | 2.11 | 15.4 |
| | | 16 | 2 | | | 1.69 | 11.5 |
| | | 24 | 8 | 1 | 1 | 0.66 | 4.4 |
| | | 30 | 2 | | | 0.02 | 0.2 |
| | | 48 | 2 | | 6 | 0.04 | 0.1 |
| *Glucosamine feeding* | Molecular glucose—equivalent: 0.370 gm. per 100 gm. of mouse | 2 | 4 | | | | 4.4 |
| | | 5 | 6 | | | 0.32 | 4.6 |
| | | 10 | 4 | | | 0.66 | 9.1 |
| | | 18 | 3 | | | 0.51 | 7.0 |
| | | 24 | 6 | 1 | | 0.21 | 2.7 |
| *Gluconate feeding* | Molecular glucose—equivalent: 0.1 gm. per 100 gm. of mouse | 5 | 4 | | | 0.45 | 23.9 |
| | | 10 | 4 | | | 0.81 | 47.5 |
| | | 20 | 6 | | | 0.35 | 18.7 |
| | | 30 | 2 | | 3 | 0.01 | 0.1 |
| *Sorbitol feeding* | Molecular glucose—equivalent: 0.1 gm. per 100 gm. of mouse | 5 | 4 | | | 0.33 | 22.0 |
| | | 10 | 2 | | | 0.44 | 25.2 |
| | | 20 | 8 | | | 0.44 | 22.2 |
| | | 30 | 2 | | | 0.05 | 2.2 |
| *Ethyl glucoside feeding (1931)* | Molecular glucose—equivalent: 0.23 gm. per 100 gm. of mouse | 2 | 5 | | | 0.24 | 3.1 |
| | | 5 | 7 | | | 0.36 | 9.0 |
| | | 10 | 3 | | | 0.46 | 13.4 |
| | | 20 | 5 | | | 0.58 | 13.1 |
| | | 30 | 4 | | | 0.51 | 11.9 |
| | | 48 | 4 | | | 0.86 | 16.7 |
| | | 72 | 2 | | | 0.35 | 8.7 |
| *Ethyl glucoside feeding (1934)* | Molecular glucose—equivalent: 0.217 gm. per 100 gm. of mouse | 5 | 4 | | | 0.51 | 13.5 |
| | | 10 | 3 | | 3 | 0.29 | 7.6 |
| | | 25 | 7 | | | 0.26 | 3.3 |
| | | 48 | 8 | | 13 | 0.30 | 5.3 |
| | | 72 | 3 | | 5 | 0.36 | 7.0 |

Figure 4 shows the percentage recovery (in the liver) of the carbohydrate fed. This figure, like figure 3, describes increases in liver glycogen; but relates it to the amount of the carbohydrate fed, rather than to the weight of fresh liver in which it was stored.

Data are also presented in table 2 to illustrate the rate of absorption as evidenced by analysis of intestinal contents.

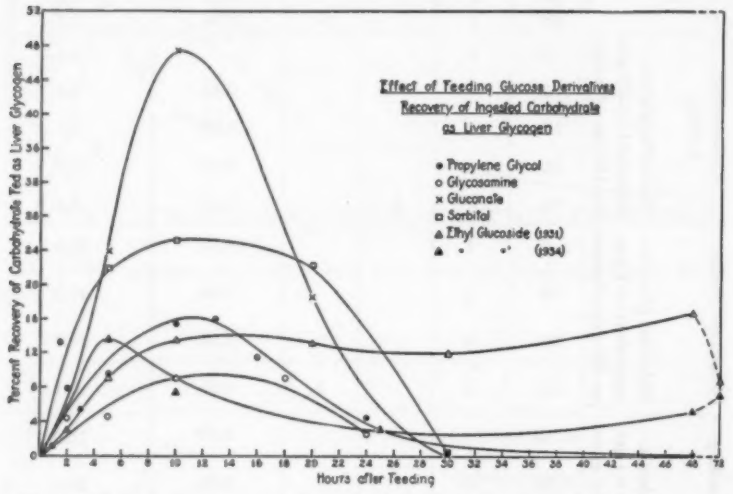


Fig. 4 These curves correspond to the data in figure 3, recalculated.

In the case of propylene glycol, the analytical method was the chromate oxidation described by Strebinger and Streit ('24) for the analysis of glycerine. The conversion factor was determined by analysis of known amounts of propylene glycol. This method is crude, because there was a large blank due to naturally occurring oxidizable matter in the intestine. The results are significant, therefore, only in indicating roughly how soon absorption was approximately finished.

## DISCUSSION

It should be emphasized that these results apply only to the history of a single carbohydrate meal administered to

fasting animals. The highest liver glycogen contents, here reported, do not attain the levels reported in the literature for continued high ingestion of carbohydrates. As might be expected, the concentration of glycogen in the liver rises higher when the glucose meal is larger.

One might expect a certain amount of glucose to be immediately burned. Indeed, our results with a very small glucose meal indicate very little storage in the liver. Above this level, however, it is not obvious why the fraction selected for deposition in the liver should remain a constant aliquot of the glucose fed.

Because of the limit imposed by gastro-intestinal tolerance, none of the substances here tested could be used in amounts as high as the maximal glucose ingestion. Nor is the storage of any of them better than that of glucose; indeed, two failed of as complete absorption as the glucose equivalent. Nevertheless, all showed undoubted evidence of glycogen storage, and its subsequent glycogenolysis as fasting proceeded. How much sugar was directly or indirectly utilized by the tissues at large was not determined in this work. It is believed, however, that the paying out of stored glycogen as fasting continued is good evidence that these substances are available as foods.

One word of caution is necessary as to the interpretation of the results. These substances were given to intact animals. The effect of intestinal action is, therefore, merged in the net effect produced. That sucrose, given parenterally, acts as a foreign substance is well recognized. It is conceivable that some of these compounds would not be utilized by an animal whose gastro-intestinal tract had been removed, even though the liver remained intact.

The effect of methyl glucoside is interesting. Its toxicity is apparent because the animals died an hour or two after feeding. Nevertheless, glycogen storage within the liver was indubitable a short time before death, as the analyses showed. The lethal effect is presumably due to progressive liberation of methyl alcohol. Similarly, the results obtained with ethyl

glucoside were erratic, presumably because of the profound anaesthetic effect produced during the first day after feeding.

No attempt has been made in this paper to review the literature pertinent to these compounds, which have not been extensively studied as sources of energy for mammals. Drury and Salter ('34) have given references to a few such investigations, most of which have been concerned with the relief of insulin hypoglycaemia, or with organ perfusions.

## SUMMARY

When the fasted mouse is fed a single large amount of appropriate carbohydrate, much of this is stored in the liver as glycogen. In the case of glucose, the maximal amount stored is roughly proportional to the amount fed, despite wide variation in the actual concentration of liver glycogen. As fasting proceeds, the stored glycogen is released from the liver. Standard curves are presented which illustrate this process in mice at various levels of glucose feeding.

For comparison, the storage and discharge of liver glycogen was followed after the ingestion of sugar derivatives in equivalent amounts. Five simple derivatives of glucose (sorbitol, gluconate, glucosamine, and the glucosides of methyl and ethyl alcohol) were studied, and one triose derivative (propylene glycol). All increased liver glycogen.

The results are compared with the effect produced by the molecular equivalent in glucose.

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# THE EFFECT OF PHOSPHATES ON THE BONES OF RACHITIC RATS

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THREE PLATES (TWELVE FIGURES)

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The earliest reference to the effect of phosphorus on bones was made by Wegner (1872). This investigator, working in Virchow's laboratory, stated that elementary phosphorus increased bone formation in growing animals and birds. Kassowitz (1884) claimed that elementary phosphorus stimulated repair of rachitic bones while Kissel (1896) reported no bone changes from the feeding of phosphorus. Phemister ('18) confirmed Wegner's (1872) and Kassowitz' (1884) observation that phosphorus was productive of bone formation. Wegner, Kassowitz and Phemister all fed elementary (red or yellow) phosphorus in daily doses of 1/100 to 1/400 of a grain. Sherman and Pappenheimer ('21) reported that the addition of alkaline potassium phosphate to a rachitiogenic diet (high in calcium and low in phosphorus) prevented rickets in rats when 0.4 per cent of the phosphate was added to, and an equivalent amount of calcium withdrawn from that diet. Hess and Unger ('22) and others have confirmed Sherman and Pappenheimer's observation. McCollum, Simmonds, and Shipley ('22 a) showed that the addition of 2 per cent of cod liver oil to a rachitiogenic diet, high in calcium and low in phosphorus, was curative of rickets in rats. Phemister, Miller and Bonar ('21) have reported that phosphorus, or phosphorus plus cod liver oil, produced similar (x-ray) results

in bone development. Two of the children fed phosphorus only in addition to their previous deficient diet during the fall and winter showed roentgenologic signs of cure of marked rickets. McCollum, Simmonds, Shipley and Park ('22 b) reported that starvation of rats, made rachitic on a high calcium low phosphorus diet, induced a cure of the rickets at the expense of other tissues of the body. Cavin ('24) reported that if rats were made to fast after having been made rachitic (on a high calcium low phosphorus diet) bone repair occurred, but convulsions and death followed. Hess and Weinstock ('26) disagreed with Wegner (1872), Kassowitz (1884) and Phemister ('18) with the statement that elementary phosphorus leads only to the production of 'phosphorus bands' at the epiphyses of the bones of rachitic rats and not to true healing. Karlitz and Shohl ('28) reported that the addition the salts of phosphorus ($Na_2HPO_4$) to a rachitiogenic diet (high in calcium and low in phosphorus), on which rats had been made rachitic, produced cure of the rickets, but in some was followed by carpopedal spasm, convulsions and death. Wilder ('28) questioned the findings of Karlitz and Shohl on the grounds that the animals studied by these authors "refused the phosphate diet to the extent of considerable loss of weight" and reported that the type of results shown can be produced by fasting as previously reported by McCollum et al. and Cavin. Lilly and Newburgh ('31) made a preliminary report that the addition of water-soluble extract of yeast and sodium phosphate ($Na_2HPO_4$) to a rachitiogenic diet (high in calcium and low in phosphorus), on which rats had been made rachitic, produced x-ray evidence of cure of the rachitic changes in such rats. Bloom ('32) reported that "the substitution of di-calcium phosphate for calcium carbonate in a rickets-producing diet resulted in the prevention and cure of rickets in albino rats." These conflicting opinions prompted us to further studies regarding the action of phosphates on rachitic rats.

## EXPERIMENTAL STUDIES

Forty-five albino rats, 28 to 31 days old, were placed in a warm, well-ventilated, dark room. These animals were from the twelfth and thirteenth generations of our own in-bred rat colony. Their weights ranged from 65 to 85 gm. They were fed Steenbock and Black ('25) rachitiogenic diet no. 2965. Distilled water was given as desired. Thirty-three days later, x-ray examinations were made. All of the animals showed advanced rachitic changes. The animals were returned to the dark room and divided into three groups.

Group I. Fifteen rats (nos. 31–45) were continued on the Steenbock-Black rachitiogenic diet no. 2965.

Group II. Fifteen rats (nos. 1–15) were fed the Steenbock-Black rachitiogenic diet no. 2965 to which 0.03 per cent of irradiated ergosterol (viosterol 250 D) by weight was added and thoroughly rubbed up in the food. This gave this diet a vitamin D (curative) value equal to that shown by McCollum, Simmonds and Shipley ('22).[1]

Group III. Fifteen rats (nos. 16–30) were fed the Steenbock-Black rachitiogenic diet no. 2965 to which sodium phosphate ($Na_2HPO_4$) was added in amounts sufficient to give the diet a 0.04 per cent value in secondary phosphorus. This is a phosphorus value sufficient to prevent rickets in rats as shown by Sherman and Pappenheimer ('21).

These diets were continued for 33 days.

For the last 33 days of the test the average gain in weight for group I was 21.5 gm. per rat; for group II, 35.5 gm. per rat; and for group III, 46.5 gm. per rat.

One animal of group I died of an undetermined cause during the experiment. No convulsions, spasms or nervous irritability was observed in any group of animals.[2]

[1] A standard viosterol (250 D) has a vitamin 'D' value 100 times that of a standard cod-liver oil.

[2] We have repeatedly observed carpopedal spasm and death in rats fed Steenbock's no. 2965, and on a few occasions convulsions, when the animals were losing weight, but have not observed these conditions when phosphates were added to the diet.

*Roentgenologic observations.* Roentgen examinations at the end of this second feeding period showed the following:

Group I. A greater x-ray evidence of rachitic changes than before.

Group II. X-ray evidence of healed rickets.

Group III. X-ray evidence of healed rickets equal to that of group II.

The entire series of rats on rachitiogenic diet no. 2965 at the end of the 33 days showed unmistakable roentgen evidence of active rickets with frayed bulbous metaphyses, lack of ossification of the growing bone with marked widening of the cartilaginous area between diaphysis and epiphysis, and some bowing of the long bones. The more marked changes, as would be expected, appeared at the wrists and knees of the animals. Similar changes were apparent at the proximal epiphyses of the humeri and femurs. The elbow was not so markedly involved, these epiphyses usually being well formed at an early period.

The group of controls which were continued on the rachitiogenic diet without corrective adjuvant showed progression of the deformities and non-ossification of the metaphyseal area. The cortical bone had not been notably disturbed. A specimen rat from this group is shown in the roentgenograms of plate 1.

The group of rats which received diet no. 2965 with irradiated ergosterol added demonstrated at the end of the 33-day period correction of the former rachitic interruption of bone growth. No marked cross striae of condensation were developed in the ends of the diaphyses. Some of the curvatures and widening of the transverse diameter of the metaphyseal portions of the shaft persisted, as would be expected. The epiphyseal growth disc again took on a smooth contour, of normal depth. The cortex of the shafts was relatively unchanged. A specimen rat from this series is shown in the roentgenograms of plate 2.

The third group which received diet no. 2965, but with the addition of sodium phosphate only, also showed at the end of the 33-day period on roentgen examination correction of

the former rachitic disturbances. In these also the epiphyseal disc had resumed a normal depth, and smoother contour, the ossification of proximate portions of the long bones apparently normal. In these animals no notable increase in density of the diaphyses in cross-striae is found. The cortical bone remained normal in architectural pattern. Some of the previous deformities in the form of curvature and increase of the diameter of the metaphyseal portions of the shafts were of course, not corrected. A specimen rat from this group is shown in the roentgenograms of plate 3.

*Chemical findings.* At the conclusion of the experiment the rats were killed and the right femur removed from several animals in each group. The bones were cleaned mechanically to remove all loose tissue and were dried at 100° for 1 week. Each bone was broken, extracted in a Soxhlet apparatus, with alcohol for 10 hours and with ether for another 10 hours, and again dried. Each bone was ashed at a dull red heat in a muffle furnace and the percentage of total ash on the moisture-free weight determined. The ash was then dissolved in a dilute hydrochloric acid, made to a convenient volume, and aliquots were used for the estimation of calcium by the method of Tisdall and Kramer ('21) and of phosphorus by the method of Fiske and Subbarow ('25). The results are presented in the following table:

*Ash of femurs*

| GROUP | ASH OF MOISTURE-FREE, FAT-FREE BONES | CALCIUM OF ASH | PHOSPHORUS OF ASH | $\frac{\text{CALCIUM}}{\text{PHOSPHORUS}}$ |
|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | |
| I | 29.4 | 38.4 | 16.0 | 2.39 |
| II | 53.4 | 40.6 | 17.3 | 2.34 |
| III | 58.7 | 39.6 | 17.7 | 2.24 |

It is evident from these data that the femurs of the phosphate-treated rats (group III) are calcified to at least as great a degree as those of the rats receiving the viosterol (group II).

*Histologic changes.* At the conclusion of the feeding period one of the femurs of a rat from each group was prepared in the Department of Pathology for microscopic examination.

Comparative photomicrographs are shown in plates 1 to 3. The animal that remained on the unmodified diet, the original no. 2965, showed the typical rachitic fragmentation of cartilage and disorganization of the bone and cartilage. Both the animal receiving irradiated ergosterol and that receiving sodium phosphate added to the rachitiogenic diet demonstrated histologic evidence of previous rachitic change. There was some persistent irregularity in alignment of the cartilage columns of the epiphyseal disc and some residual fragmentation. In detail, however, the zone of primary calcification, the cartilage columns, and the resting cartilage had resumed normal proportions and a normal character of individual cells in both of the treated rats. The trabeculae of the shaft proximal to the zone of primary calcification in the phosphate specimen did not show appreciable difference from the irradiated ergosterol specimen.

The depth of osteoid tissue proximal to the epiphyses in the untreated animal was in striking contrast to the lack of a broad osteoid zone and the marked organization of new trabeculae in both treated specimens.

It is of note that the roentgen evidence of apparent cure is not completely supported by the histology of the tissue.

From the histologic standpoint the addition of basic sodium phosphate to the rachitiogenic diet corrects a rachitic animal equally as well as the administration of irradiated ergosterol.

## DISCUSSION

Particular comment has been made above on the resumption of normal architectural pattern of the epiphyseal discs and the metaphyseal portions of the shafts without demonstrable sclerosis. Previous observers, notably Hess and Weinstock ('26), have called attention to a band of increased density of bone in a shaft which they attributed to phosphorus (the 'phosphorschicht'). This appeared, however, with the use of yellow phosphorus. Phemister ('18) also described a zone of increased longitudinal trabeculae in the ends of the shafts. It is of note that Phemister and his associates ('21) reported a complete correction of rickets in two severe cases by the addition to the previous dietary of 1/100 to 1/150 grain of phosphorus thrice daily. In contrast to the observations of Hess, our series of experimental animals which received sodium phosphate did not show 'phosphorus bands.'

Further, changes in the character of the cortical bone were described by earlier observers with the administration of phosphorus. We did not find such to be the case in the experimental series.

## CONCLUSIONS

Rickets was produced in white rats by a diet high in calcium and low in phosphorus. The subsequent addition of phosphates to this diet, caused x-ray, chemical, and histological evidences of healing, at least equal to that caused by the addition of irradiated ergosterol (viosterol) to the diet of another group of rats previously made rachitic in the same manner. No neuropathic symptoms were noted in any animal of any group, and no 'phosphorus bands' were present in any specimen.

We wish to express our appreciation of the advice of Dr. L. H. Newburgh and the assitance of Dr. C. V. Weller during this experiment.

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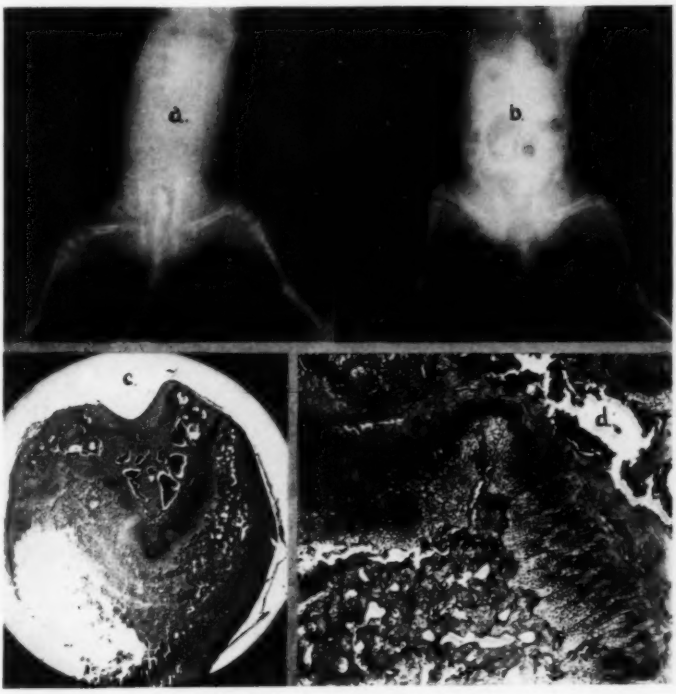


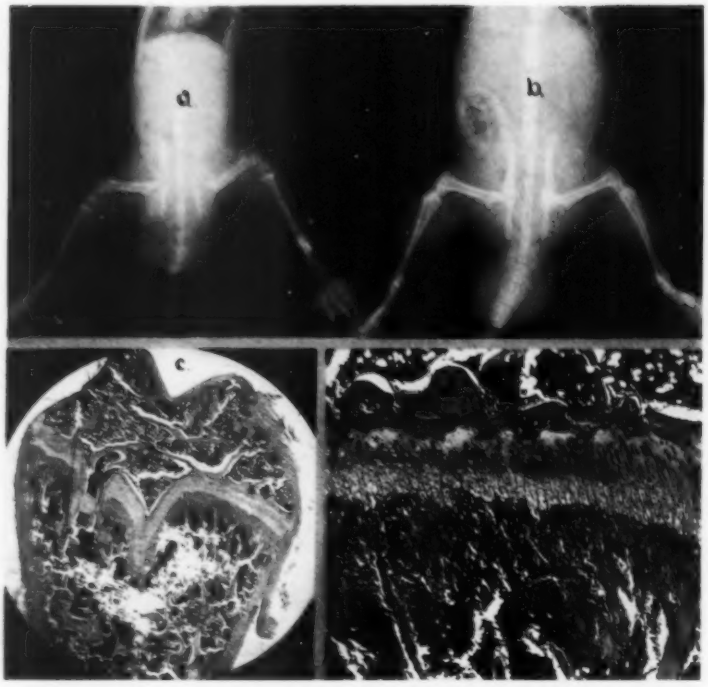

Rat no. 37 (group I) control, on diet 2965 only.

a Roentgenogram after diet 2965 for 33 days (total age 64 days), active rickets.

b Roentgenogram after diet 2965 for 66 days (total age 97 days), progressive rachitic change.

c Photomicrograph (of longitudinal section of distal end of femur), showing deformity of epiphyseal cartilage zone, with increased depth, broad band of osteoid tissue proximal to cartilage.

d Photomicrograph (detail), showing depth and irregularity of cartilage, poor primary ossification, numerous osteoid trabeculae with poor marrow development.



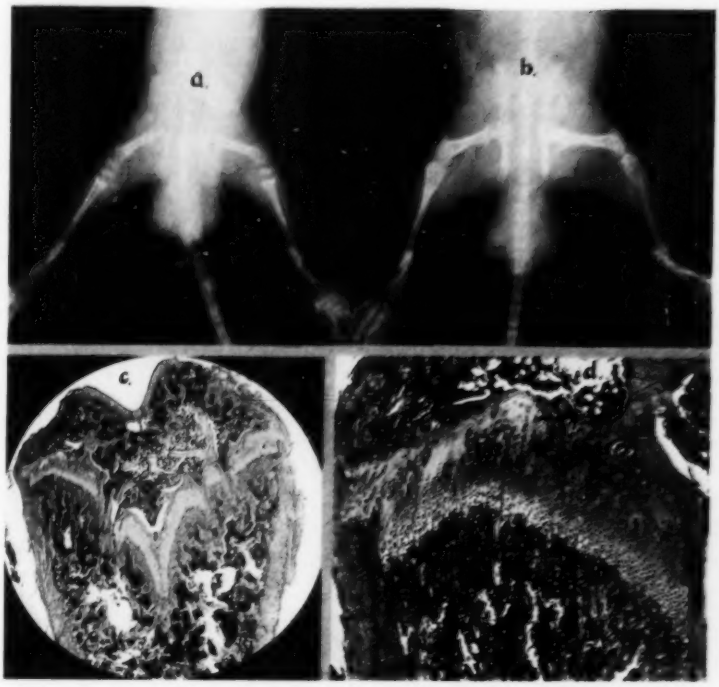

Rat no. 6 (Group II) diet 2965 only for 33 days, irradiated ergosterol (0.03 per cent by weight) added for 33 days.

a Roentgenogram at end of first period on rachitiogenic diet. Comparable changes to that of the specimen of group I.

b Roentgenogram at end of second or corrective period. X-ray evidence of cure of previous growth disturbance of epiphyseal junction.

c Photomicrograph of longitudinal section of femur (distal end). Residual irregularity of line of cartilaginous zone. But more normal depth than in group I. Normal depth of primary zone of calcification. No marked condensation of longitudinal trabeculae.

d Photomicrographic detail—good alignment and character of cartilage, normal zone of primary calcification, normal development of marrow spaces.




Rat no. 26 (group III) diet 2965 for 33 days plus sodium phosphate ($Na_2HPO_4$) for 33 more days.

a Roentgenogram at end of first period (diet 2965 only). Active rachitic changes at growth zones.

b Roentgenogram at end of second period (diet 2965 plus $Na_2HPO_4$) x-ray evidence of cure of rickets. Resumption of normal architecture at growing zones.

c Photomicrograph—longitudinal section of distal end of femur—residual irregularity of epiphyseal disc line—normal depth of zones—little variation from specimen of group II.

d Photomicrographic detail. No greater number of longitudinal trabeculae in metaphysis than in group II. No broad osteoid zone. Active growth of medullary area. Good resumption of normal cell alignment.

# CATARACT IN RATS FED ON HIGH LACTOSE RATIONS

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TWO FIGURES

(Received for publication June 30, 1934)

The occurrence of nutritional cataract in rats has been reported previously, but the etiological factors are not well established in spite of several contributions to the subject. At least three groups of workers have associated lens changes with a vitamin G deficiency. Day, Langston and O'Brien ('31), Langston and Day ('33) and Day and Langston ('34) conclude that cataract, conjunctivitis, and keratitis develop in gray or albino rats deprived of vitamin G in nearly 100 per cent of the cases. O'Brien ('32) and Yudkin ('33) have obtained cataract in animals deprived of vitamin G at an early date, whereas no cataract has developed in older animals. The latter concludes that at the present stage of our knowledge "we are not justified in attributing the formation of cataract to the lack of G alone." He reports furthermore that rats showed inflammatory and other ocular changes accompanying opacity of the crystalline lens. Curtis, Hauge and Kraybill ('32) have found lens opacities apparently due to a tryptophane deficiency and make no mention of vitamin G as a significant factor. A recent paper by Chaikoff and Lachman ('33) reports the occurrence of cataract associated with long-standing experimental diabetes in depancreatized dogs in which the ration had apparently been adequately supplied with protein and vitamins. The lens changes are attributed to

the fact that the diabetic condition had existed over a considerable period of time.

The various types of experimental cataract reported by these workers do not seem to point to any common etiological factors. The data reported in this paper suggest a metabolic disturbance rather than a vitamin or amino acid deficiency as the cause of the cataractous changes. Mature or markedly advanced cataracts have occurred in all rats fed a synthetic laboratory ration in which excessively high levels of lactose were the sole source of carbohydrate. Control animals fed on starch, maltose, dextrin or sucrose, respectively, as the only carbohydrate, in an otherwise similar ration, have never developed any ocular abnormalities. The accidental observation of mature cataract in the first three rats which had received a 70 per cent lactose ration in connection with another experiment stimulated the present investigation.

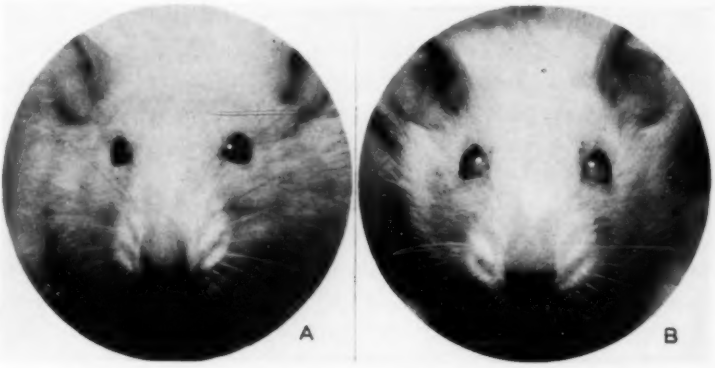
## PROCEDURE

The rations consisted of 70 per cent carbohydrate, 15 per cent casein, 5 per cent (Osborne and Mendel) salt mixture, 9 per cent Crisco and 2 per cent cod liver oil fed ad libitum plus adequate daily doses of vitamin B complex in the form of 0.5 gm. dry brewer's yeast fed separately. Special precautions were taken to reinforce both the vitamin A and G supply to avoid these as complicating factors.

Rats on the 70 per cent lactose ration receiving four times the usual dose of dry brewer's yeast (2 gm. daily) developed cataract as soon as control rats from the same litter receiving the usual amount of yeast (0.5 gm. daily). Furthermore, there was no evidence at any time of a vitamin G deficiency. Growth was slightly subnormal on the 70 per cent lactose ration, but normal on the lower levels. All control rats on the other sources of carbohydrate grew normally with the 0.5 gm. of yeast as a daily supplement. The rats on the lactose rations appeared as sleek and well nourished as the control rats aside from the fact that they were blind. Young animals when first put on the ration often had a diarrhea

which later subsided, although the stools frequently continued to be softer than those of rats on other carbohydrates. This condition might partially account for the retarded growth in young rats fed the 70 per cent lactose ration. The very lack of other striking abnormalities is in itself a significant finding.

Lest the lactose from one source might contain some unknown contaminant, a C.P. grade from another source was used in a limited number of cases with exactly the same results as those obtained with the less expensive grade of the sugar.




A, Normal rat. B, rat with cataract resulting from a 70 per cent lactose ration.

Beta-lactose was also used for comparison with the regular commercial form with the similar outcome. Thus it would appear that lactose, per se, must have been the significant factor in all cases.

Weekly ophthalmoscopic examinations were made on the eyes of both experimental and control animals. During this routine procedure the observer purposely remained ignorant of the ration of the specific animals, in order to avoid any unconscious prejudice in reporting findings. A detailed description of the ophthalmoscopic appearance of these lens opacities is significant. Oftentimes the earliest lens changes were noticed from one to several weeks before distinct

opacities were observed and recorded. In tabulating results those animals which were not examined early, or those which were not recorded when first observed, are considered separately.

The development of cataractous changes may show considerable variation in type. The progressive lens opacities may begin first as a zone of defraction, a fine line in the lens around the periphery of the nucleus. As the beam of light is shifted by moving the ophthalmoscope from one position to another, the zone of defraction appears in one place and disappears in another. By this means the nucleus of the lens can be outlined in its entirety. The second step in this process is an increase in the size, width and density of this zone of defraction. This progresses with greater or lesser rapidity until there is often a ring of opacity outlining the entire nucleus of the lens, followed by a complete opacity of the nucleus if the progress is continuous.

A second form of lens change often found is a cortical opacity developing in the zonular region. This is located under the anterior lens capsule. The cortical opacities appear most frequently in the region of the ciliary body. First, one or several discrete flake opacities appear which frequently progress and coalesce until the entire lens is opaque. In a few of the eyes observed this opaque area appeared as a fine lace work, especially in those animals which had received an inceased amount of sodium chloride in the diet.

A third form of cataract development is a combination of the first and second. A zone of defraction develops and sooner or later cortical opacities also appear, or vice versa. The process may then continue to complete opacity or remain immature. This combination is by far the most common form of opacity development. In fact, those confined to cortical or nuclear portions of the lens alone are infrequent. In determining the type of opacity the ophthalmoscopic appearance alone was the criterion, no use being made of the slit lamp or the microscope.

Histological preparations were made of a few typical specimens by the celloidin method and stained with hematoxylin and eosin. The microscopic picture of the cataractous lens is typical. The microscopic appearance of the cornea, sclera, ciliary body and retina is normal, the same as that of the normal control eyes. Thus there is no evidence of the type of pathology usually associated with a vitamin A deficiency.

## RESULTS

The summary of findings given in table 1 shows the number of mature and immature cataracts occurring in each ration

TABLE 1

*Incidence of cataract in rats on different types and amounts of carbohydrates*

| RATION GROUPS | NUMBER OF RATS | AGE WHEN STARTED | PROGRESS OF CATARACT DEVELOPMENT | | | | | | | PERCENTAGE SHOWING | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | First appearance | | Mature cataract | | Immature cataract | | None | Any lens changes | Mature cataract |
| | | | Number | Weeks | Number | Weeks | Number | Weeks | | | |
| | | *days* | | | | | | | | | |
| 70 % lactose | 49 | 26 | 18<br>31 | 5.0[1]<br>1.3 | 33 | 10.0 | 16 | 22 | 0 | 100 | 68 |
| 70 % lactose | 4 | 60 | 4 | 8.0 | 2 | 15.5 | 2 | 31 | 0 | 100 | 50 |
| 70 % lactose + Ca gluconate | 5 | 26 | 5 | 2.4 | 3 | 13.0 | 2 | 12 | 0 | 100 | 60 |
| 50 % lactose | 26 | 25 | 13<br>13 | 8.0[1]<br>2.4 | 7 | 17.0 | 18 | 34 | 1 | 96 | 27 |
| 50 % lactose | 2 | 60 | 1 | 13.0 | | | 1 | 31 | 1 | 50 | 0 |
| 30 % lactose | 6 | 23 | 6 | 3.6 | | | 6 | 37 | 0 | 100 | 0 |
| 30 % lactose | 2 | 60 | | | | | | | 2 | 0 | 0 |
| 70 % starch | 16 | 27 | | | | | | | 16 | 0 | 0 |
| 70 % dextrin | 14 | 25 | | | | | | | 14 | 0 | 0 |
| 70 % maltose | 4 | 23 | | | | | | | 4 | 0 | 0 |
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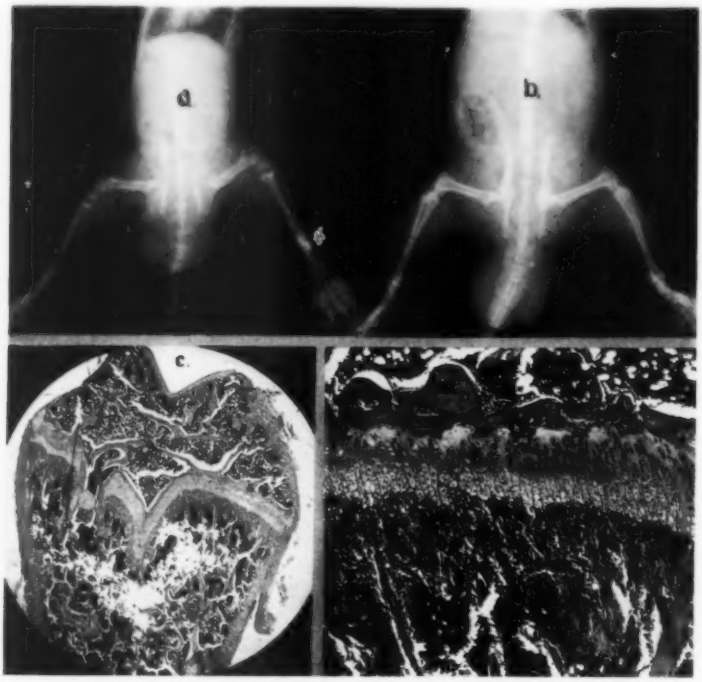


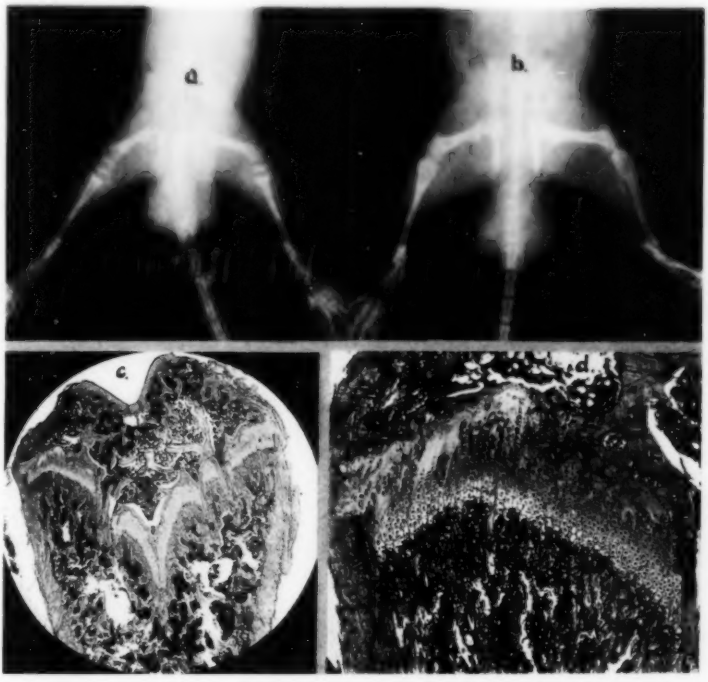

Rat no. 6 (Group II) diet 2965 only for 33 days, irradiated ergosterol (0.03 per cent by weight) added for 33 days.

a Roentgenogram at end of first period on rachitiogenic diet. Comparable changes to that of the specimen of group I.

b Roentgenogram at end of second or corrective period. X-ray evidence of cure of previous growth disturbance of epiphyseal junction.

c Photomicrograph of longitudinal section of femur (distal end). Residual irregularity of line of cartilaginous zone. But more normal depth than in group I. Normal depth of primary zone of calcification. No marked condensation of longitudinal trabeculae.

d Photomicrographic detail—good alignment and character of cartilage, normal zone of primary calcification, normal development of marrow spaces.




Rat no. 26 (group III) diet 2965 for 33 days plus sodium phosphate ($Na_2HPO_4$) for 33 more days.

a Roentgenogram at end of first period (diet 2965 only). Active rachitic changes at growth zones.

b Roentgenogram at end of second period (diet 2965 plus $Na_2HPO_4$) x-ray evidence of cure of rickets. Resumption of normal architecture at growing zones.

c Photomicrograph—longitudinal section of distal end of femur—residual irregularity of epiphyseal disc line—normal depth of zones—little variation from specimen of group II.

d Photomicrographic detail. No greater number of longitudinal trabeculae in metaphysis than in group II. No broad osteoid zone. Active growth of medullary area. Good resumption of normal cell alignment.

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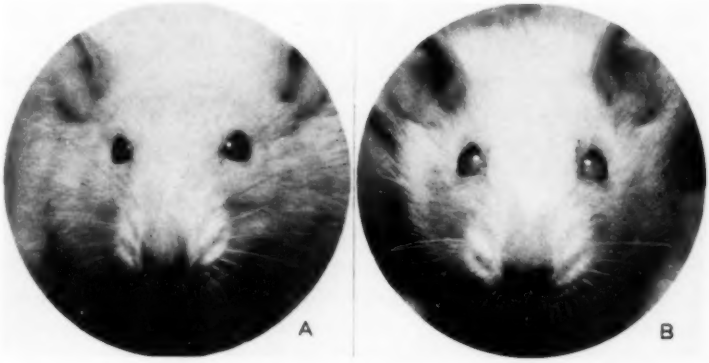


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group and the average number of weeks required for the development of these changes. Rats on the 70 per cent lactose ration showed progressive cataractous changes in all animals leading to mature bilateral cataract in 68 per cent of the cases. Rations containing lesser quantities of lactose resulted in slower development or a less pronounced type of lens change. Thus only 27 per cent of the rats on 50 per cent lactose developed mature cataract and none on the 30 per cent lactose ration. Of those developing mature cataract the average time required was 10 weeks on the 70 per cent ration and 17 weeks on the 50 per cent ration.

Practically all rats on the various lactose rations showed some lens changes. If those animals are disregarded which were not examined weekly from the beginning of the experiment there is a striking comparison between the level of lactose fed and the time required for the first appearance of early lens changes, namely, 1.3, 2.4 and 3.6 weeks, respectively, on the 70 per cent, 50 per cent and 30 per cent lactose rations. The lower the level of lactose fed, the longer was the time required for the development of lens changes.

This high incidence of cataract on the 70 per cent lactose ration and the literature relevant to the effect of lactose on calcium metabolism suggest several lines of investigation which would throw further light on this problem. An intensive preliminary study was made on a small group of animals: Twelve rats on the 70 per cent lactose ration and six controls were divided into five groups, as shown in table 2 and as many data as possible were collected on these groups. For calcium determinations on blood serum and eyes it was necessary to combine groups I and II and groups III and IV in order to have enough material for analysis. Two 24-hour urine samples from each rat were collected and tested for reducing sugar during the week before the animals were sacrificed. The Folin-Wu micro method was employed for urinary sugar and modified slightly for blood sugar determinations. Non-fasting blood sugars were high and irregular. The rats in the first five groups had had free access to food

immediately before blood was taken for sugar determinations. Subsequent work on groups VI, VII and VIII showed that 12 hour fasting blood sugars ran much lower and entirely within normal limits for both the lactose and control groups. The urinary sugar was high in all of the groups fed on lactose; but group V, which had developed immature cataract showed less sugar than those with mature cataract. This observation on so small a group may not be significant. Further studies are in progress on the nature of the sugar excreted. Micro-chemical methods and osazone tests alone have proved unsatisfactory as means of differentiation.

TABLE 2

*Metabolic changes associated with cataract*

| GROUPS AND CARBOHYDRATE CONTENT OF RATIONS | | NUMBER OF RATS | FINAL AGE | FINAL BODY WEIGHT | INCIDENCE OF CATARACT | REDUCING SUGAR | | ASH IN FAT-FREE FEMURS | CALCIUM CONTENT OF EYES MILLIGRAMS PER 100 GM. | | SERUM CALCIUM MILLIGRAMS PER 100 CC. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Urine milligrams per 24 hours | Blood [1] milligram per 100 cc. | | Moist | Dry | Total | Diffusible |
| | | | *weeks* | *gm.* | | | | *per cent* | | | | |
| I | 70 % starch | 3 | 24 | 192 | None | 2.7 | 122 | 65.4 | 8.4 | 35.0 | 10.5 | 4.5 |
| II | 70 % maltose | 3 | 39 | 283 | None | 5.7 | 95(2) | 65.2 | | | | |
| III | 70 % lactose | 4 | 30 | 148 | Mature 17 weeks | 34.2 | 177(3) | 66.5 | 16.1 | 78.0 | 10.6 | 3.7 |
| IV | 70 % lactose | 4 | 17 | 113 | Mature 8.5 weeks | 39.5 | 135(3) | 61.5 | | | | |
| V | 70 % lactose | 4 | 32 | 212 | Immature at end of 29 weeks | 18.9 | 160 | 67.0 | 11.0 | 48.0 | 12.2 | 4.0 |
| VI | 70 % starch | 8 | 30 | 232 | None | | 88.0 | | | | | |
| VII | 70 % lactose | 15 | 31 | 190 | Mature and immature | | 74.8 | | | | | |
| VIII | 50 % lactose | 14 | 35 | 257 | Mature and immature | | 76.2 | | | | | |

[1] Groups I–V blood sugar determinations made without a preliminary fast

Groups VI–VIII blood sugar determinations made after a 12-hour fast

Qualitative or quantitative reducing sugar determinations before and after controlled fermentation periods have indicated the presence in the urine of a slowly fermentable sugar, such as galactose. The quantity of urine in young rats has made it impossible to run quantitative tests in all cases. These results plus the experience of other investigators yield exidence of galactosuria as a frequent result of high lactose ingestion.

Some of the data on calcium metabolism also seem to be significant. Total serum calcium and diffusible calcium do not show much deviation from the normal, although more determinations and on larger groups of animals should be made before definite conclusions are drawn. The calcium content of the cataractous eyes is about twice the normal, while that of eyes with immature cataract show less of an increase. That this was not merely a dehydration effect is shown by figures given on both moist and dry basis. Total ash of dry fat-free femurs was determined to see whether any significant bone changes were associated with this pathological condition. The constant high per cent ash in the bones of all groups indicates no significant effect there. This finding is not contradictory to that of Kline et al. ('32) on rachitic chicks, because our rats were not rachitic and calcification beyond the physiological optimum was not to be expected.

## DISCUSSION

Inasmuch as high blood sugar in a diabetic is known to cause certain temporary lens changes and to predispose the older diabetic to cataract (O'Brien, '32 a), there appears to be a relationship between an excess sugar in the blood and the cataratous changes in the eyes. There is no evidence available regarding the influence of a specific sugar on the lens structure, but it is quite possible that traces of a foreign sugar in the blood, such as lactose or galactose, may have as detrimental an influence as an excess of glucose. McCance and Madders ('30) and Cori ('25) have shown that rats absorb galactose more rapidly than other sugars, while Folin and

Berglund ('22) have previously demonstrated a rapid excretion due to the lack of a renal threshold for galactose or lactose. Numerous other investigators have shown that these two sugars are unique in many of their metabolic processes. Further investigation as to the nature and extent of blood sugar changes in these animals will be significant.

It has been demonstrated by Bergeim ('26) and Kline et al. ('32) that lactose causes an increased absorption of calcium and possibly phosphorus from the intestinal tract. This specific influence of lactose on calcium absorption or upon its utilization has been repeatedly demonstrated in connection with the relief of parathyroid tetany (Dragstedt and Peacock, '23; Inouye, '24; Greenwald and Gross, '29). Tetany was relieved by lactose plus calcium salts, whereas calcium alone failed. It has also a decided favorable effect upon the calcification of rachitic bones in chicks (Kline et al., '32). Through its influence on the intestinal flora, this sugar is known to promote a relatively high pH through the entire length of the intestinal tract. The increased permeability of the intestinal membrane for certain minerals seems to be greater, however, than can be accounted for on the basis of the increased acid reaction of the intestine (Robinson and Duncan, '31; Sherman and Hawley, '22).

The logical result of the increased absorption would be a serum calcium higher than normal. If, however, a mere increase in blood calcium were the result of the lactose acting in the intestine one should be able to simulate this condition by intravenous injection of calcium salts. Such experiments have been tried on parathyroidectomized dogs without success (Adams, '29). Robinson et al. ('29) showed calcium retention from bone meal to be doubled by the use of lactose and concludes: "It seems plausible that lactose. . . . exerts a specific action in facilitating the passage of calcium into the blood stream, acting in this respect independently of the pH of the intestines." The fact that serum calcium was raised by calcium salts, but that calcium retention and the relief of tetany resulted only when lactose was also used indicated that

there must have been some difference in the availability of the calcium circulating in the blood stream. The recent paper of Benjamin and Hess ('33) lends weight to such an hypothesis.

The inorganic constituents of cataractous human lenses have been investigated by Grabar and Nordman ('33), MacKay et al., ('32), Updegraff, ('32), Salit ('33) and Kirby ('30). It is significant that in all of these analyses the cataractous lenses contained more calcium than the normal, while the potassium was distinctly lower than normal in most cases; in fact, there was some alteration in proportion of several of the inorganic elements. In rat experiments the small size makes it more convenient to use whole eyes rather than the lenses for analyses. For this reason the calcium figures given in table 2 are less striking than they would be if less extraneous tissue had been included.

Changes in osmotic pressure which might result from an increased amount of sugar in the blood could scarcely account for this extensive migration of inorganic ions. If, however, the sugar increases the permeability of the delicate capsular membrane, such a shift in ions might be possible. This theory is supported by the observation that the mineral composition of the cataractous lens is much more nearly similar to the mineral composition of blood plasma than is that of a normal lens. Thus, if the normal protective action of the capsular membrane is destroyed, free exchange between plasma, aqueous humor and the lens substance might take place. It is well known that a puncture of the capsular membrane which admits aqueous humor will produce cataract. Thus the protoplasmic substance of the lens is apparently held in perfect solution by a nice balance of the inorganic ions present. If this balance is disturbed the proteins might be precipitated with the production of observed opacities.

The question as to whether animals other than rats are susceptible to cataract as a result of lactose feeding was investigated with kittens and rabbits. No lens changes were seen in any of these animals. The kittens developed such

a severe diarrhea that the amount of lactose had to be reduced. The rabbits showed no lens abnormalities in 16 weeks on their skim milk-lactose ration in which the sugar constitutes 70 per cent of the dry weight. That rats may be peculiarly susceptible to cataract is suggested by the fact that lens changes occur in rats with a vitamin G deficiency, while cataract is not a characteristic symptom of a similar deficiency associated with pellagra in man.

The quantities of lactose used would never be approached in any but a laboratory diet. This fact coupled with the apparent susceptibility of the rat to cataractous development makes it impossible to apply these findings directly to the human or other animal species. On the other hand, the experimental production of cataract and its control by dietary means in a lower animal may become a valuable device for the more detailed study of cataractous development and accompanying metabolic changes.

## SUMMARY

1. Mature bilateral cataract has occurred in 68 per cent of rats fed on a 70 per cent lactose ration with advanced lens changes showing in 100 per cent of the animals.

2. Rations containing lower quantities of lactose resulted in slower development or less pronounced type of lens change.

3. Cataracts have never appeared in rats fed starch, maltose, dextrin or sucrose, respectively, as the sole source of carbohydrate at the 70 per cent level.

4. The progressive lens changes have been described in detail. The developing opacities give the histological picture of human cataract.

5. Blood, urine, and tissue analyses on a limited number of animals indicated some disturbance in both carbohydrate and mineral metabolism with a loss of galactose in urine.

6. The calcium content of cataractous eyes doubles the normal. The total and diffusible calcium content of blood serum did not seem to be significantly altered.

7. The possibility of producing or controlling the development of cataract in lower animals by dietary means promises to be a valuable device for further and more detailed study of the etiology of cataract.

We are much indebted to Dr. Helen Sternberger, of the Children's Fund of Michigan, for calcium determinations on blood and eyes.

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# THE EFFECT OF FERTILIZERS AND SOIL TYPES ON THE MINERAL COMPOSITION OF VEGETABLES [1]

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Considerable interest recently has been manifested in the mineral content of food crops. This is especially true of such elements as calcium, iron, copper and manganese. In Florida there has long been a question as to whether the rather infertile soils produced crops low in mineral content or whether the heavy fertilization with commercial fertilizers produced crops having a high content of minerals. In order to throw some light on this subject, analyses of truck crops grown under known conditions of fertilizer treatment and on known soil types in various sections of Florida were undertaken.

The analyses reported in this paper were made largely on vegetables obtained from fertilizer experiments conducted in various sections of the State. As soon as possible after receipt of the samples they were washed and the edible portion was cut into small pieces and dried in an oven at about 75° C. When dry they were ground in a Quaker mill to a fine powder and stored in glass containers, tightly stoppered. All analyses are reported on a moisture-free basis.

The chemical methods employed were largely those of the American Association of Official Agricultural Chemists with

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some minor modifications. Copper was determined by the Biazzos method as modified by Elvehjem.

The figures for iron content are probably high, due to the fact that all of the materials were ground in an iron mill. At the start of these experiments the chief interest was in the other minerals rather than iron, hence no special precautions were taken in preparing the samples to avoid contamination with iron.

Table 1 (A) gives the analyses of tomatoes from plots differently fertilized. The first analysis shows the composition of tomatoes grown with a normal fertilizer application; the second, with a fertilizer containing only one-half the amount of phosphoric acid; the third, with a fertilizer containing a double amount of potash. Comparing the first two analyses, the only difference is in the phosphoric acid content, there being a slightly lower percentage where less was used in the fertilizer. Comparing the first and third analyses, no differences are found, especially none in the potash content, even though twice as much potash fertilizer was applied to those vegetables used in the third analysis. The last analysis shows the composition of tomatoes when the usual superphosphate in the fertilizer was omitted and colloidal phosphate, a very finely divided raw phosphate, was used as the source of phosphoric acid. In comparing this analysis with the first one, a lower content of phosphoric acid and of potash is found. As the phosphoric acid in the colloidal phosphate is less readily available than that in superphosphate, this may account for the lower phosphate content in the vegetables analyzed.

Table 1 (B) shows the analyses of lettuce variously fertilized. While there are some variations in the analyses, none can be attributed to the differences in the fertilizers used. As was the case with the tomatoes, changing the potash ratio in the fertilizer had no influence on the potash absorbed by the lettuce. The colloidal phosphate, even when used in double quantity, did not influence the mineral composition.

TABLE 1

*Effect of fertilizers on composition of crops*

| FERTILIZER TREATMENT | ASH | Cl | $SO_3$ | $P_2O_5$ | $SiO_2$ | N | $Na_2O$ | $K_2O$ | CaO | MgO | Fe PPM | Mn PPM | Cu PPM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A. Tomatoes** | | | | | | | | | | | | | |
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | | | |
| Normal fertilizer | 10.65 | 0.43 | 0.78 | 1.81 | 0.16 | 2.80 | 0.28 | 6.25 | 0.32 | 0.53 | 123 | 50 | 15 |
| ½ $P_2O_5$ | 10.25 | 0.46 | 0.81 | 1.59 | 0.18 | 2.69 | 0.16 | 6.18 | 0.29 | 0.50 | 193 | 44 | 11 |
| 2 × $K_2O$ | 10.45 | 0.51 | 0.84 | 1.74 | 0.12 | 2.70 | 0.32 | 6.13 | 0.30 | 0.50 | 172 | 47 | 10 |
| Colloidal $P_2O_5$ | 10.39 | 0.50 | 0.67 | 1.60 | 0.13 | 2.84 | 0.30 | 5.99 | 0.30 | 0.49 | 214 | 52 | 12 |
| **B. Lettuce** | | | | | | | | | | | | | |
| Normal fertilizer | 17.09 | 1.31 | 1.23 | 1.74 | 1.00 | 3.59 | 0.34 | 7.75 | 1.75 | 0.20 | 480 | 177 | 29 |
| ½ $K_2O$ | 17.01 | 1.82 | 1.28 | 1.67 | 1.00 | 3.56 | 0.40 | 8.03 | 1.67 | 0.25 | 481 | 226 | 25 |
| 2 × $K_2O$ | 16.70 | 1.55 | 1.61 | 1.71 | 1.00 | 3.47 | 0.40 | 7.10 | 1.85 | 0.28 | 470 | 208 | 30 |
| 3 × $K_2O$ | 17.93 | 1.80 | 1.24 | 1.73 | 1.00 | 3.64 | 0.40 | 8.14 | 1.83 | 0.24 | 475 | 203 | 26 |
| Colloidal $P_2O_5$ | 16.11 | 1.44 | 1.47 | 1.83 | 1.00 | 3.64 | 0.40 | 7.28 | 1.84 | 0.18 | 430 | 185 | 29 |
| 2 × colloidal $P_2O_5$ | 16.72 | 1.31 | 1.42 | 1.88 | 1.00 | 4.09 | 0.40 | 7.57 | 1.74 | 0.23 | 471 | 209 | 30 |
| **C. Celery** | | | | | | | | | | | | | |
| Phosphoric acid | 17.19 | 1.31 | 2.56 | 1.56 | 0.24 | 3.93 | 3.02 | 2.29 | 4.52 | 0.79 | 163 | 15 | 300 |
| Potash | 22.23 | 2.27 | 2.14 | 1.44 | 0.04 | 4.05 | 3.01 | 5.71 | 4.38 | 0.72 | 135 | 8 | 300 |
| Phosphate and potash | 21.68 | 1.70 | 2.64 | 1.49 | 0.05 | 3.61 | 2.37 | 4.87 | 4.67 | 0.76 | 150 | 18 | 293 |
| Nitrogen, phosphate and potash | 21.50 | 1.70 | 2.45 | 1.45 | 0.04 | 3.77 | 2.72 | 5.30 | 4.40 | 0.74 | 185 | 23 | 200 |
| No fertilizer | 18.66 | 1.73 | 2.27 | 1.25 | 0.14 | 3.91 | 2.90 | 2.51 | 4.41 | 0.77 | 130 | 13 | 413 |
| Nitrogen, phosphate, potash and sulfur | 19.97 | 1.45 | 2.78 | 1.38 | 0.03 | 3.69 | 2.44 | 5.00 | 3.91 | 0.69 | 130 | 30 | 140 |
| Triple fertilizer application | 19.22 | 1.24 | 2.26 | 1.82 | 0.08 | 4.08 | 1.59 | 6.27 | 3.41 | 0.67 | 240 | 10 | 150 |

Table 1 (C) shows the analyses of celery grown on Everglades muck soils with and without fertilizers. Comparing the celery grown on no-fertilizer plots with that grown on the phosphoric acid treated plots, there is a slight increase in sulfates and phosphoric acid in the fertilized celery. These increases are hardly significant, however, as there is almost as high a phosphoric acid content in the crop on the plot receiving only potash, and a lower sulfate content where a triple fertilizer application was made. Comparing the unfertilized celery with the potash-treated, a marked increase in the potash content is found; in fact, more than double the amount. Comparing the celery receiving phosphate and potash, the potash content is still high, but not quite so high as where potash alone was used. The only difference between the celery produced on the complete fertilizer plots and that from the no-fertilizer plots is in the higher potash content wherever this material was used. Tripling the fertilizer application brought about a slightly greater increase in potash and phosphoric acid. The copper content varies considerably. This is undoubtedly due to the heavy applications of Bordeaux spray and the inability to remove all traces of copper sulfate in preparing the samples for analyses.

In connection with some collaborative work with the American Association of Official Agricultural Chemists on the availability of phosphates, some interesting results were obtained. Soy beans were grown in quartz sand to which a nutrient solution without phosphoric acid was added. The phosphate was added separately in the form of superphosphate, mono-calcium phosphate and various ammoniated phosphates, at a basic rate equivalent to 400 pounds per acre of 18 per cent superphosphate. The results are shown in table 2. It will be noted that there was very little difference in the yield of dry materials. The phosphate analyses, however, show interesting differences. Taking first the superphosphate, a marked increase in phosphate content is found in soy beans which had received increased applications of phosphate fertilizer. The same is true where mono-calcium

phosphate was the source of phosphoric acid. In the case of the ammoniated phosphates, decreasing amounts of phosphoric acid are taken up as the percentage of ammonia in the phosphate increases. The availability of the phosphoric acid in ammoniated superphosphate, as determined chemically, decreases in the same order.

## INFLUENCES OF SOIL TYPES

The following tables show the effect produced by different soil types on the composition of vegetables grown on them.

As many readers may not be familiar with the soil types mentioned in the following pages a brief description of their

TABLE 2

*Effect of various phosphatic fertilizers on phosphate content of soy beans*

| SOURCE OF $P_2O_5$ | DRY WEIGHT IN GRAMS | PER CENT $P_2O_5$ $H_2O$ FREE |
|---|---|---|
| Superphosphate, ½ standard | 5.5 | 0.47 |
| Superphosphate, standard | 6.5 | 0.82 |
| Superphosphate, 2 × standard | 6.0 | 1.34 |
| Ammoniated superphosphate, 2.48 per cent $NH_3$ | 6.5 | 0.62 |
| Ammoniated superphosphate, 5.36 per cent $NH_3$ | 7.0 | 0.50 |
| Ammoniated superphosphate, 6.83 per cent $NH_3$ | 7.0 | 0.37 |
| Ammoniated superphosphate, 8.66 per cent $NH_3$ | 7.0 | 0.29 |
| Mono-calcium phosphate, ½ standard | 8.25 | 0.35 |
| Mono-calcium phosphate, ½ standard | 7.75 | 0.51 |
| Mono-calcium phosphate, standard | 8.50 | 0.93 |
| Mono-calcium phosphate, 2 × standard | 7.25 | 1.88 |

general characteristics is given together with a table showing their average nitrogen, phosphoric acid and potash content.

Bladen soil is normally a poorly drained soil containing a fair amount of organic matter and of plant nutrients. It is considered the best Irish potato soil in the State and is also used extensively for truck crops. The Glade marl is, as its name implies, made up largely of finely divided marl (90 to 95 per cent calcium carbonate) overlain with a layer of mucky material from 3 to 12 inches deep. These lands are usually flooded during the summer and early fall, and thus undergo a thorough leaching each year. The Hernando soils are among

the more fertile soils in the State, containing slightly more plant food and organic matter than do the Bladen.

The Coxville soils are very similar to the Bladen, but have somewhat better natural drainage and a more plastic subsoil. The Norfolk soils are the predominating soils of the high lands of the State. They are low in humus or organic matter and low in plant nutrients. The Fellowship soils are very similar to the Hernando soils. The Leon soils are naturally among the poorest in the State. They are underlain with a hardpan layer and are low in plant foods. However, when sub-irrigation and drainage systems are installed and heavy fertilizer applications made, they produce bountiful crops.

Saw-grass muck soils are the predominating type found in the Everglades. They are soils in which the organic matter has not been decomposed very much. Technically speaking, they are peats rather than mucks. They are not productive when first put into cultivation unless given special treatment with salts of copper, manganese and zinc. The Parkwood soils are poorly drained soils underlain with marl. They produce excellent crops when drained, as they are among the richest soils in plant food that we have in the State.

TABLE 3

*Content of nitrogen, phosphorus and potassium in soil types*

| SOIL | NITROGEN (N) | PHOSPHORIC ACID ($P_2O_5$) | POTASSIUM OXIDE ($K_2O$) |
|---|---|---|---|
| Bladen | 0.107 | 0.027 | 0.048 |
| Hernando | 0.198 | 0.080 | 0.047 |
| Norfolk | 0.050 | 0.019 | 0.011 |
| Leon | 0.087 | 0.007 | 0.009 |
| Parkwood | 0.272 | 0.462 | 0.054 |
| St. Lucie | 0.025 | 0.012 | 0.006 |

Table 4 (A) shows analyses of tomatoes grown on Bladen, Glade marl and Hernando soil types. The differences in composition of the tomatoes are not great. The tomatoes grown on Hernando soil have a higher ash content and higher chlorine and sodium content, undoubtedly due to a higher sodium chloride content in the soil which lies near the coast.

TABLE 4

*Effect of soil type on composition of crops*

| SOIL TYPE | ASH | Cl | $SO_3$ | $P_2O_5$ | $SiO_2$ | N | $Na_2O$ | $K_2O$ | CaO | MgO | Fe PPM | Mn PPM | Cu PPM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A. Tomatoes** | | | | | | | | | | | | | |
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | | | |
| Bladen | 11.00 | 0.48 | 0.80 | 1.73 | 0.13 | 2.81 | 0.29 | 6.17 | 0.31 | 0.51 | 159 | 51 | 11 |
| Glade marl | 11.72 | 0.79 | 0.88 | 1.49 | 0.02 | 3.71 | 0.25 | 7.06 | 0.48 | 0.61 | 171 | 70 | 15 |
| Hernando | 12.64 | 0.91 | 0.78 | 1.85 | 0.55 | 3.04 | 0.61 | 6.51 | 0.63 | 0.51 | 133 | 28 | 22 |
| **B. Potatoes** | | | | | | | | | | | | | |
| Coxville | 5.04 | 0.22 | 0.60 | 0.68 | 0.20 | 1.45 | 0.24 | 2.45 | 0.31 | 0.35 | 94 | 27 | 13 |
| Bladen | 5.27 | 0.47 | 0.59 | 0.76 | 0.13 | 1.16 | 0.27 | 2.66 | 0.32 | 0.31 | 76 | 21 | 22 |
| Norfolk | 5.40 | 0.39 | 0.58 | 0.61 | 0.15 | 1.46 | 0.19 | 3.14 | 0.33 | 0.38 | 69 | 11 | 10 |
| Fellowship | 4.46 | 0.41 | 0.74 | 0.69 | 0.09 | 2.45 | 0.23 | 2.24 | 0.29 | 0.36 | 65 | 10 | 10 |
| Leon | 5.41 | 0.43 | 0.71 | 0.90 | 0.37 | 2.15 | 0.20 | 2.88 | 0.26 | 0.29 | 71 | 11 | 20 |
| **C. String beans** | | | | | | | | | | | | | |
| Muck | 7.88 | 0.43 | 0.92 | 1.03 | 0.56 | 4.31 | 0.19 | 2.92 | 1.33 | 0.72 | 145 | 23 | 25 |
| St. Lucie | 7.74 | 0.42 | 0.85 | 1.53 | 0.15 | 3.30 | 0.27 | 2.89 | 1.39 | 0.65 | 146 | 51 | 13 |
| Parkwood | 7.00 | 0.50 | 0.93 | 1.11 | 0.29 | 3.38 | 0.25 | 1.80 | 0.90 | 0.09 | 168 | 22 | 14 |
| **D. Cabbage** | | | | | | | | | | | | | |
| Leon | 8.74 | 0.44 | 2.52 | 1.52 | 0.26 | 3.01 | 0.31 | 3.50 | 1.14 | 0.05 | 160 | 31 | 21 |
| Saw-grass muck | 9.00 | 0.92 | 2.60 | 1.55 | 0.29 | 2.55 | 0.25 | 4.00 | 1.60 | 0.04 | 120 | 28 | 11 |
| Coxville | 8.05 | 0.46 | 2.69 | 1.17 | 0.14 | 2.78 | 0.44 | 3.84 | 0.81 | 0.04 | 280 | 34 | 15 |
| Parkwood | 10.06 | 0.74 | 2.27 | 1.28 | 0.12 | 3.16 | 0.30 | 4.42 | 1.83 | 0.04 | 154 | 29 | 10 |

It is worthy of note that tomatoes grown on marl had a lower calcium content than those grown on Hernando soil. The lower amount of phosphoric acid in the tomatoes grown on marl is probably due to the high pH of this soil which depresses the availability of the phosphoric acid. The higher manganese content of the marl tomatoes is due to the use of manganese in the fertilizer.

Table 4 (B) shows the analyses of potatoes grown on various soil types. In comparing these analyses very little differences are found. The chief difference is in the nitrogen content of the potatoes grown on the Fellowship and Leon soils. Just why these should be so much higher is difficult to explain. The higher manganese content of the potatoes grown on the Coxville and Bladen soils is due to the inclusion of manganese in some of the fertilizers used on these plots.

Table 4 (C) gives the analyses of string beans growing on Everglades muck, St. Lucie sand and Parkwood sandy loam. The St. Lucie soils are about the poorest soils from an agricultural standpoint that we have in the State, being very low in natural fertility and in organic matter or humus. However, analyses of beans growing on this infertile soil show that when fertilized it produces crops having as high a mineral content as when grown on the rich Everglades muck soil, and higher than when grown on Parkwood soils which naturally are more fertile soils.

Table 4 (D) shows cabbage grown on a variety of soil types. Here again the differences in composition are not outstanding, with one or two exceptions. The cabbage grown on muck and Parkwood soils carries more chlorine. Both of these soils are located on the east coast, while the other two soils are located in the central section of the State. The phosphoric acid content is somewhat lower in cabbage grown on the Coxville and Parkwood soils; nitrogen is higher in cabbage produced on Leon and Parkwood, while calcium is very low in the Coxville cabbage.

The claim has been made that Florida grown vegetables contain a higher water content than those grown farther north

and that, therefore, it takes more of them to supply the same amount of nutrients. Table 5 shows the moisture content of vegetables grown in Florida compared with those reported by Peterson and Elvehjem ('28). With the exception of strawberries and beets, the agreement between the two sets of figures is remarkably close.

As was stated in the beginning of the paper, there has been considerable doubt in the minds of many as to the mineral content of truck crops grown in Florida. In table 6 are shown comparisons of the mineral content of Florida grown vege-

TABLE 5

*Moisture content of vegetables*

| | FLORIDA | PETERSON |
|---|---|---|
| | *Per cent* | *Per cent* |
| Beans | 90.89 | 91.40 |
| Carrots | 89.33 | 90.10 |
| Cabbage | 92.73 | 92.60 |
| Celery | 92.54 | 94.00 |
| Lettuce | 94.83 | 94.40 |
| Potatoes | 79.76 | 78.20 |
| Tomatoes | 95.20 | 94.20 |
| Beets | 88.85 | 82.30 |
| Peppers | 93.68 | 94.00 |
| Strawberries | 94.01 | 90.30 |
| Turnips | 91.76 | 91.50 |

tables with analyses reported by Sherman ('32). In comparing these analyses it must be borne in mind that the analyses reported by Sherman were made a good many years ago. Refinements in the methods of analyses probably account for some of the differences. This is undoubtedly true of the figures for sulfur. A remarkable feature of this table is the close agreement in almost every case of the figures for potash. In the majority of cases Florida grown crops have a higher mineral content than those shown in Sherman's tables. This is especially true of calcium and phosphoric acid, two elements that are receiving considerable attention from nutrition specialists.

The last table, 7, compares the copper and manganese found in vegetables grown in Florida with that found by other workers. The Florida analyses were, with one exception,

TABLE 6

*Ash constituents in fresh materials in per cent of edible portion*

| | CaO | MgO | $K_2O$ | $Na_2O$ | $P_2O_5$ | $SO_3$ | Cl | |
|---|---|---|---|---|---|---|---|---|
| Beans (string) | 0.064 | 0.041 | 0.298 | 0.026 | 0.119 | 0.075 | 0.024 | Sherman |
| | 0.115 | 0.053 | 0.281 | 0.024 | 0.124 | 0.081 | 0.040 | Florida |
| Beets | 0.041 | 0.035 | 0.425 | 0.125 | 0.089 | 0.040 | 0.058 | Sherman |
| | 0.074 | 0.060 | 0.440 | 0.159 | 0.123 | 0.087 | —— | Florida |
| Cabbage | 0.063 | 0.025 | 0.298 | 0.036 | 0.066 | 0.165 | 0.024 | Sherman |
| | 0.083 | 0.004 | 0.266 | 0.022 | 0.116 | 0.183 | 0.032 | Florida |
| Carrots | 0.078 | 0.035 | 0.346 | 0.136 | 0.105 | 0.055 | 0.036 | Sherman |
| | 0.099 | 0.047 | 0.472 | 0.091 | 0.157 | 0.093 | 0.057 | Florida |
| Celery | 0.109 | 0.023 | 0.381 | 0.113 | 0.085 | 0.055 | 0.156 | Sherman |
| | 0.328 | 0.055 | 0.395 | 0.203 | 0.108 | 0.183 | 0.013 | Florida |
| Lettuce | 0.060 | 0.028 | 0.408 | 0.036 | 0.096 | 0.035 | 0.074 | Sherman |
| | 0.090 | 0.010 | 0.400 | 0.018 | 0.090 | 0.064 | 0.068 | Florida |
| Peppers | 0.008 | 0.017 | 0.167 | —— | 0.060 | 0.035 | 0.013 | Sherman |
| | 0.031 | 0.027 | 0.280 | —— | 0.078 | 0.061 | 0.025 | Florida |
| Potatoes | 0.020 | 0.046 | 0.517 | 0.028 | 0.133 | 0.075 | 0.038 | Sherman |
| | 0.061 | 0.068 | 0.540 | 0.047 | 0.148 | 0.129 | 0.077 | Florida |
| Strawberries | 0.057 | 0.032 | 0.177 | 0.067 | 0.064 | 0.035 | 0.006 | Sherman |
| | 0.049 | 0.051 | 0.202 | 0.022 | 0.062 | 0.043 | 0.019 | Florida |
| Tomatoes | 0.015 | 0.017 | 0.331 | 0.013 | 0.060 | 0.035 | 0.034 | Sherman |
| | 0.038 | 0.044 | 0.542 | 0.022 | 0.139 | 0.068 | 0.038 | Florida |
| Turnips | 0.090 | 0.028 | 0.407 | 0.075 | 0.105 | 0.162 | 0.041 | Sherman |
| | 0.137 | 0.049 | 0.353 | 0.096 | 0.114 | 0.167 | 0.091 | Florida |
| Eggplant | 0.015 | 0.025 | 0.169 | 0.013 | 0.078 | 0.040 | 0.024 | Sherman |
| | 0.031 | 0.035 | 0.265 | 0.035 | 0.105 | 0.055 | 0.057 | Florida |
| Onions | 0.048 | 0.027 | 0.214 | 0.022 | 0.103 | 0.175 | 0.021 | Sherman |
| | 0.097 | 0.035 | 0.269 | 0.041 | 0.130 | 0.181 | 0.038 | Florida |

made of crops that had received no manganese or copper as fertilizer. The exception was celery, which had all been fertilized with copper sulfate. Of course, practically all of the

vegetable crops had been sprayed several times with Bordeaux mixture, which may account in part for the higher copper

TABLE 7

*Manganese and copper content of vegetables*
*(ppm. in moisture free material)*

| | Mn | | | | Cu | | |
|---|---|---|---|---|---|---|---|
| | Lindow and Peterson | Remington and Shiver | Richards | The authors | Lindow Elvehjem Peterson | Remington and Shiver | The authors |
| Beets | 35.1–76.2 | 55.4 | 21.7 | 23.5 | 11.5 | 9.1 | 10–25 |
| Cabbage | 10.6 | 40.7 | 15.0 | 23–58 Av. 30 | 6.8 | 6.3 | 10–28 Av. 17.8 |
| Carrots | 6.0 | 42.2 | 16.7 | 25–40 | 8.1 | 10.7 | 10–20 |
| Celery | 27.1 | | | 8–67 Av. 32 | 2.0 | | 100–1600 |
| Beans (string) | | 35.2 | | 20–25 | 12.0 | 9.6 | 7–20 |
| Eggplant | | 31.1 | | 40–50 | 13.6 | 12.7 | 7–16 |
| Lettuce | 216 N 100 S | 118.4 | 133 | 141–345 Av. 197 | 11.6 | 10.9 | 18–33 Av. 28.6 |
| Onions | 7.9 | 63.3 | 9.8 | 50–62 | 13.4 | 11.5 | 13–16 |
| Peppers | 18.2 | | | 13–70 | 16.1 | | 8–18 |
| Potatoes (Irish) | 4.5 | 9.4 | 14.3 | 5–21 Av. 13.7 | 8.0 | 7.4 | 4–28 Av. 16.4 |
| Potatoes (sweet) | 5.4 19.6 | 9.3 | | 8 | 5.2 | 6.2 | 8 |
| Tomatoes | | 25.7 | | 20–65 Av. 48 | 9.9 | 17.4 | 6–24 Av. 11.7 |
| Turnips | | 13.6 | 3.6 | 9.5 | 11.0 | 4.4 | 14 |

content. Both copper and manganese are fully as abundant in vegetables grown in Florida as in those grown in other sections of the country. We found, as others have found,

that the content of copper and manganese varies greatly in samples of the same vegetable grown in different sections of the State. The content of both of these elements can be increased through the use of manganese sulfate or copper sulfate in the fertilizer.

It will be extremely interesting to study the composition of crops produced through the continued use of the new synthetic and highly concentrated fertilizers. Considering the small amount of some of the secondary plant nutrients contained in these fertilizers, and the small amounts of these elements present in Florida soils, the time may come when this combination will bring about a lowered mineral content of the crops and also a lowered yield. The results reported in this paper indicate the following:

## SUMMARY

1. That complete fertilizers containing nitrogen, phosphoric acid and potash, when used in the usual amounts necessary for optimum crop production exert very little influence on the composition of the crops grown with them.

2. Heavy applications of fertilizer will nullify any effects which soil types may have on the composition of the crop.

3. Florida grown vegetables are as high in minerals, including copper and manganese, as vegetables produced in other sections of the country; in some cases their mineral content is higher than that of vegetables from elsewhere.

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# THE STABILITY OF VITAMINS B($B_1$), G($B_2$) AND $B_4$ [1]

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ONE FIGURE

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A study of the heat stability of vitamins B($B_1$) and G($B_2$) in natural grain diets using the chick as the experimental animal has been reported by Elvehjem, Kline, Keenan and Hart ('32). With the knowledge of the intense requirement of this species for vitamin $B_4$ as reported by Keenan, Kline, Elvehjem and Hart ('33) it was decided to extend our knowledge to the heat stability of this factor and to investigate further the stability of the antineuritic and antipellagric components of the vitamin B complex.

We have tested the stability of these factors in a natural grain diet, in a commercial yeast, and in a hog's liver preparation. It is our purpose in this paper to discuss, 1) the heat stability of vitamins B, G and $B_4$ in the foodstuffs mentioned; 2) the separation of these factors by differential heat treatment, and from this knowledge, 3) the formulation of basal diets suitable for assaying foodstuffs for their antineuritic, antipellagric, or antiparalytic properties.

## EXPERIMENTAL

White Leghorn chicks were used exclusively as experimental animals. The basal grain diet, and the method used for housing and feeding these birds throughout the experimental period was the same as that previously described by Elvehjem,

[1] Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

Kline, Keenan and Hart ('32). The yeast (baker's) was obtained fresh from the Red Star Yeast Company in Milwaukee and dried at 65°C. before using. The liver was a vacuum desiccated product obtained from the Wilson Laboratories, Chicago, and designated as 'special whole liver substance (Wilson).'

The heat treatments of the grain ration, yeast, and whole liver substance were all made at their natural hydrogen ion concentration. The pH is therefore constant, the medium, degree, and time of heating being the variables. The presence of moisture in the medium such as obtains in the autoclave, by heating a substance in the fresh state or by moistening a previously dried foodstuff is designated as treatment in a moist medium. The dry medium entails the treatment of a dry or relatively dry foodstuff in an electrically heated oven.

## THE STABILITY OF VITAMIN B($B_1$)

When the natural grain diet is fed to chicks they attain an average growth of 320 to 350 gm. by the sixth week. Autoclaving this diet, however, inactivates the vitamin B completely and polyneuritis is demonstrable in 7 to 9 days. With the incorporation of 4 per cent of the dried Red Star yeast or 18 per cent whole liver substance into the autoclaved diet, a growth is obtained comparable with that of the untreated ration. The heated liver and yeast were incorporated into the autoclaved ration at these same levels and at higher levels to determine the degree of destruction.

Table 1 has been compounded from a series of determinations made upon these foodstuffs subjected to various heat treatments. Complete inactivation of the vitamin is indicated by the negative symbol, whereas one, two or three positive signs have been used to demonstrate the relative degree of destruction which is determined by the deviation from the normal growth curve.

In the previous paper by Elvehjem, Kline, Keenan and Hart ('32) the observation was made that the antineuritic factor was stable in the grain ration when heated dry at

100°C. for 1 day, but if this treatment was prolonged for 6 days some inactivation did occur. More vitamin G is inactivated in this process, however, for symptoms of pellagra are observed. Raising the temperature to 110°C. and maintaining this treatment for 6 days does not cause a greater destruction of vitamin B. At 120°C. This factor is stable for a period of 5 hours, is slightly destroyed if the treatment is prolonged for a day, and completely inactivated at this temperature for 6 days.

TABLE 1

*The heat stability of vitamin B($B_1$)*

| SUBSTANCE TESTED | | pH | HEAT TREATMENT | | | VITAMIN B STABILITY |
|---|---|---|---|---|---|---|
| | | | Medium | Temperature | Hours | |
| Ration | | 5.5–6.0 | Moist | Autoclave | 5 | — |
| Yellow corn | 58 | 5.5–6.0 | Dry | 100°C. | 144 | ++[1] |
| Wheat middlings | 25 | 5.5–6.0 | Dry | 110°C. | 144 | ++[1] |
| Casein | 12 | 5.5–6.0 | Dry | 120°C. | 5 | +++ |
| NaCl | 1 | 5.5–6.0 | Dry | 120°C. | 24 | ++[1] |
| | | 5.5–6.0 | Dry | 120°C. | 144 | — |
| Yeast | | 6.0–6.5 | Dry | 120°C. | 24 | ++ |
| Yeast | | 6.0–6.5 | Moist | Autoclave | 5 | — |
| Yeast | | 6.0–6.5 | Dry | 120°C. | 144 | — |
| Liver | | 6.0–7.0 | Moist | 100°C. | 24 | — |
| Liver | | 6.0–7.0 | Dry | 100°C. | 24 | +++ |
| Liver | | 6.0–7.0 | Dry | 120°C. | 24 | ++ |

[1] Symptoms of pellagra.

The antineuritic potency of yeast and liver is lowered slightly by a dry heat treatment of 120°C. for 24 hours. The previous observation that heating fresh yeast at 100°C. for 1 day would inactivate the vitamin B may now be extended to liver. The antineuritic value of the liver and yeast may be retained, however, by drying at 65°C. prior to heating at 100°C. The vitamin B in the ration, yeast and liver is therefore completely inactivated by autoclaving and destroyed to a large extent in yeast and liver by heating fresh at 100°C. for 24 hours. However, in the absence of moisture, the tem-

perature may be increased to 120°C., with very little destruction.

### THE STABILITY OF VITAMIN G($B_2$)

The natural grain diet which has been heated to 100°C. for 144 hours has been used as a basal diet in assaying the yeast and liver for their antipellagric properties. Chicks reared upon this diet will develop symptoms of pellagra in 3 weeks. The incorporation of 2 per cent yeast or 1 per cent whole liver

TABLE 2

*The heat stability of vitamin G*

| SUBSTANCE TESTED | pH | HEAT TREATMENT | | | VITAMIN G STABILITY |
|---|---|---|---|---|---|
| | | Medium | Temperature | Hours | |
| Ration | 5.5–6.0 | Dry | 100°C. | 144 | — |
| Yellow corn 58<br>Wheat middlings 25<br>Casein 12<br>NaCl 1 | 5.5–6.0 | Dry | 120°C. | 24 | — |
| Yeast | 6.0–6.5 | Dry | 100°C. | 144 | + |
| Yeast | 6.0–6.5 | Dry | 120°C. | 24 | — |
| Yeast | 6.0–6.5 | Moist | 100°C. | 24 | +++ |
| Yeast | 6.0–6.5 | Moist | Autoclave | 5 | +++ |
| Liver | 6.0–7.0 | Dry | 100°C. | 144 | + |
| Liver | 6.0–7.0 | Dry | 120°C. | 24 | — |
| Liver | 6.0–7.0 | Moist | Autoclave | 5 | +++ |
| Liver | 6.0–7.0 | Moist | Autoclave | 10 | ++ |

substance with this ration will supply all requirements of the chick for this factor.

From table 2 it will be observed that there is as great a destruction of the vitamin G in the ration by heating at 120°C. for 24 hours as is accomplished by the 144-hour heating at 100°C. The latter heating process inactivates approximately 50 per cent of the vitamin G contained in yeast and liver. However, a very large part of the antipellagric factor may be inactivated in the yeast and liver by the heat treatment of 120°C. for 1 day—a process which does not alter the vitamin B content appreciably. For this procedure, the grain ration

is placed in small pie pans in layers about $\frac{1}{2}$ inch thick. When the electric oven is filled with the ration, approximately 5 hours is required for the temperature to attain 120°C., so that the ration is heated only 19 hours at this temperature.

### THE STABILITY OF VITAMIN $B_4$

The vitamin $B_4$ activity of the ration and liver was tested by incorporating them into a synthetic diet which we have shown to be deficient in this factor (Keenan, Kline, Elvehjem and Hart, '33). It has been found recently that autoclaving the casein of this diet will remove the last remaining traces of the antiparalytic factor which makes the ration a more drastic one for $B_4$ assay studies. This synthetic ration which carries the autoclaved casein is designated diet no. 441A. The yeast, because of its very low antiparalytic activity, was not assayed. Table 3 designates the various heat treatments to which the grain ration and liver were subjected.

The inclusion of 32 per cent of the natural grain diet (ration 240) into the synthetic ration renders this diet complete for the chick. The grain diet which has been heated to 100°C. for 6 days has been used as a basal G low diet. Examination of table 3 indicates that all but a small portion of the vitamin $B_4$ is destroyed by this treatment. This emphasizes the inadequacy of this diet in testing a foodstuff for its antipellagric properties because of its deficiency in the antiparalytic factor. However, treatment of the basal diet at 120°C. for 24 hours does not alter the vitamin $B_4$ content appreciably, although it is extremely low in vitamin G, hence its applicability for vitamin G assay studies.

It will be observed (table 3) that there is some inactivation of the vitamin $B_4$ in dried whole liver by moistening and again drying at 65°C. for 24 hours. Autoclaving the liver completely destroys the vitamin $B_4$ contained therein, but by subjecting the natural grain diet to this treatment the greater portion of this factor is retained. It is therefore apparent that the presence of moisture catalyzes the destruction of vitamin $B_4$ as well as that of vitamin B, as shown by Elvehjem, Kline, Keenan and Hart ('32) yet in the autoclave both the

ration and liver are heated in an equivalent medium. This suggests the greater lability of vitamin $B_4$ in liver as compared to grains when treated under similar conditions.

TABLE 3

*The heat stability of vitamin $B_4$*

| RATION NO. | NUMBER CHICKS | SUPPLEMENT | HEAT TREATMENT OF SUPPLEMENT | AVERAGE WEIGHT 6 WEEKS | NUMBER WITH PARALYSIS | DEATHS AT 6 WEEKS | VITAMIN $B_4$ ACTIVITY |
|---|---|---|---|---|---|---|---|
| 441 A | 7 | None | None | | 7 | 7 | — |
| 441 A | 7 | 32 per cent ration 240 | None | 365 | 0 | 0 | +++ |
| 441 A | 7 | 32 per cent ration 240 | 100°C.—144 hours (G low diet) | ... | 6 | 5 | + |
| 441 A | 7 | 32 per cent ration 240 | Autoclave—5 hours (B low diet) | 255 | 3 | 0 | ++ |
| 441 A | 7 | 32 per cent ration 240 | 120°C.—24 hours dry (G low diet) | 322 | | | ++ |
| 441 A | 7 | 18 per cent whole liver | | 350 | | | +++ |
| 441 A | 2 | 18 per cent whole liver | Moistened and dried at 65°C.—24 hours | 315 | | | ++ |
| 441 A | 7 | 18 per cent whole liver | Moistened and dried at 100°C.—24 hours | | 7 | 7 | — |
| 441 A | 7 | 18 per cent whole liver | Autoclaved—5 hours | | 7 | 7 | — |

THE DIFFERENTIAL DESTRUCTION OF VITAMINS B, G AND $B_4$

A comparison of the heat stability of these factors is given in table 4, which embodies the observations which have been made on the grain ration, the yeast, and the liver. Using a moist medium, all the factors are relatively stable at 65°C. for 24 hours. When the temperature is increased to 100°C. for the same length of time the antineuritic and antiparalytic components are inactivated. Autoclaving the ration inactivates vitamin B, but a greater portion of vitamin G and $B_4$ are retained. Autoclaving liver or yeast, however, destroys the antineuritic and antiparalytic factors, but saves vitamin G.

Using a dry heat all factors are stable at a temperature of 100°C. maintained for 24 hours. Prolonging this treatment

for 6 days inactivates vitamins G and $B_4$ but the antineuritic factor is relatively stable. A treatment of 120°C. for 24 hours will inactivate G completely and retain the greater portion of vitamins B and $B_4$.

Thus vitamin B may be destroyed and the greater portion of vitamin G and $B_4$ retained by autoclaving the grain ration for 5 hours at 15 pounds' pressure. Vitamin G is inactivated and the other fractions preserved in the grain ration by a dry heat treatment of 120°C. for 24 hours. A portion of the antiparalytic potency of liver is lost by moistening and drying at 65°C. for 24 hours.

TABLE 4

*Heat stability of vitamins B, G and $B_4$*

| HEAT TREATMENT | | | VITAMIN G | VITAMIN B | VITAMIN $B_4$ |
|---|---|---|---|---|---|
| Medium | Temperature | Hours | | | |
| Moist | 65°C. | 24 | +++ | +++ | ++ |
| Moist | 100°C. | 24 | — | +++ | — |
| Moist | Autoclave (ration) | 5 | — | +++ | ++ |
| Moist | Autoclave (liver) | 5 | — | +++ | — |
| Dry | 100°C. | 24 | +++ | +++ | +++ |
| Dry | 100°C. | 144 | ++ | + | + |
| Dry | 120°C. | 24 | ++ | — | ++ |
| Dry | 120°C. | 144 | — | — | — |

## BASAL DIETS USED IN ASSAYING FOODSTUFFS FOR VITAMINS B, G AND $B_4$

In view of these results we have modified the diets originally proposed for B and G studies. Autoclaving the ration as previously indicated (table 3) destroys some of the antiparalytic factor. To cover this deficiency, 2 per cent of whole liver substance is added. The composition of the diet (ration 242A) now used for B assay studies is as follows:

| | | |
|---|---|---|
| Yellow corn | 57 | |
| Wheat middlings | 25 | Autoclaved 5 hours |
| Crude casein | 12 | 15 pounds' pressure |
| NaCl | 1 | |
| $Ca_3(PO_4)_2$ | 1 | |
| $CaCO_3$ | 1 | |
| Special whole liver substance (Wilson) | 2 | |
| Cod liver oil | 1 | |

The 2 per cent $CaCO_3$ has been changed to 1 per cent $CaCO_3$ and 1 per cent $Ca_3(PO_4)_2$ and the cod liver oil reduced from 2 to 1 per cent to make this part of the ration comparable to the method for vitamin D assay in vitamin D carriers for poultry by Hart, Kline and Keenan ('31).

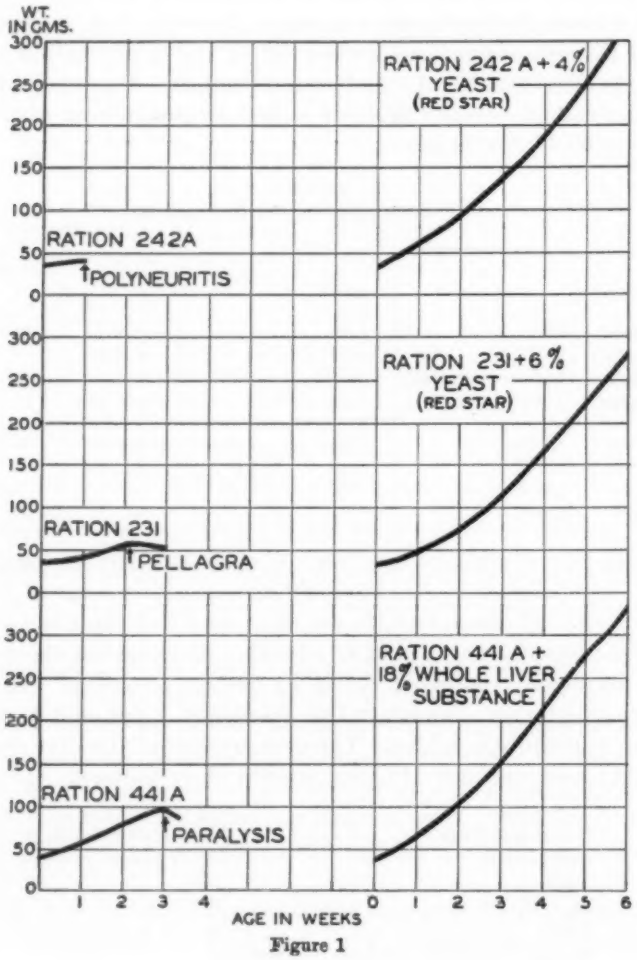


Figure 1

Typical growth curves of chicks confined to this basal diet only and with 4 per cent yeast addition are given in figure 1.

The inadequacy of the basal grain diet for vitamin G studies, which has been heated to 100°C. for 144 hours and the superiority of the ration treated at 120°C. for 24 hours has been discussed. Not only does this diet contain more vitamin $B_4$, but it is more deficient in the antipellagric factor than the diet previously proposed. The composition of the basal diet now used for G assay studies (ration 231) is

| | | |
|---|---|---|
| Yellow corn | 59 | Heated 120°C. for 24 hours |
| Wheat middlings | 25 | |
| Crude casein | 12 | |
| NaCl | 1 | |
| $CaCO_3$ | 1 | |
| $Ca_3(PO_4)_2$ | 1 | |
| Cod liver oil | 1 | |

The performance of birds confined to this diet alone and with a 6 per cent yeast supplement is given in figure 1.

The basal vitamin $B_4$ deficient diet used for studies on this factor is the same as that previously described with the exception that the casein is autoclaved. It has been designated ration no. 441A and has the composition—

| | |
|---|---|
| Dextrin | 53.5 |
| Autoclaved casein | 24.0 |
| Salts | 2.5 |
| Yeast (Anheuser Busch) | 8.0 |
| Autoclaved liver residue | 10.0 |
| Cod liver oil | 2.0 |

The incidence of the abnormality in chicks confined to this diet is very uniform. The behavior of birds confined to the basal diet no. 441A and with 18 per cent whole liver is given in figure 1.

## DISCUSSION

As a result of the knowledge gained from a supply of the stability of vitamins B, G and $B_4$, basal diets have been formulated which may be used in testing the distribution of these factors in foods. Although the heat treatments utilized in these studies are not comparable, in general, with the methods used in the process of preservation and preparation of human foods, they are, however, quite similar to methods used by commercial companies in the preparation of animal foods.

These studies emphasize the relative lability of vitamin $B_4$. This fact, combined with the limited supply of this component in human dietaries substantiates the contention, previously made, "that at least this factor may be a critical one in normal nutrition."

Our knowledge concerning the chemistry of the vitamin B complex has increased considerably during the past 3 years. Windaus, Tschesche and Ruhkopf ('32), Ohdake ('34), and others have shown that their antineuritic concentrates contain sulphur in the molecule. It is possible that the presence of this element renders the molecule more labile. That the inactivation may be one of hydrolysis is suggested from the fact that degradation is catalyzed by the presence of moisture in the heating medium.

Kuhn, György and Wagner-Jauregg ('33) have correlated vitamin G activity with the flavine pigments obtainable from milk, eggs, or liver and bearing the empirical formula $C_{17}H_{20}O_6N_4$. It is difficult to visualize what happens to the pigment when such foodstuffs as mentioned above are subjected to treatments which will render them deficient in this factor. It is our opinion that further work is necessary to substantiate the view that the antipellagric factor is an integral part of the pigment molecule.

With the exception of Tschesche's ('33) report that Reader's vitamin $B_4$ concentrate (Barnes, O'Brien and Reader, '32), was a substance very closely allied with adenine, little is known concerning the chemistry of this factor. Barnes, O'Brien and Reader ('32) have prepared concentrates

of this factor by methods which are somewhat similar to the methods used for the preparation of vitamin B, with one exception, that of differential adsorption upon charcoal. Vitamin B is adsorbed at a pH of 7 whereas vitamin $B_4$ is selected preferentially by the adsorbent at a pH of 1. This demonstrates the proximity in chemical characteristics of vitamins B and $B_4$. The results presented in this paper demonstrate the similarity in heat stability of the antineuritic and antiparalytic components. We suggest, therefore, that the molecule of vitamin B is possibly very similar to that of vitamin $B_4$ and the antiparalytic factor may be inactivated by the same process which functions in the degradation of vitamin B.

Although autoclaving the grain diet inactivates the greater portion of the vitamin B, vitamin $B_4$ is comparatively stable. Autoclaving liver inactivates both the antineuritic and antiparalytic factors. This emphasizes the influence of surrounding or accompanying material on the rate of $B_4$ destruction. The similarity in heat stability of vitamins B and $B_4$ in yeast and liver raises the question as to whether or not some of the abnormalities which have been ascribed to the polyneuritic syndrome may not be due in part at least to a deficiency of vitamin $B_4$.

## CONCLUSIONS

1. Vitamin B($B_1$) in yeast, liver, and in a natural grain ration was destroyed completely by autoclaving and inactivated to a great extent in yeast and liver by heating in the fresh state at 100°C. for 24 hours.

2. Vitamin G($B_2$) may be inactivated in the foodstuffs tested by a dry heat treatment of 120°C. maintained 24 hours. Vitamin B is relatively stable to this thermal treatment. Under such conditions vitamin G does not conform to the conception that it is the more heat stable factor.

3. Vitamin $B_4$ is similar in heat stability to that of vitamin B. This suggests that much of the past work with vitamin B deficiency may be due, in part, to a deficiency of $B_4$.

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# A STUDY OF THE IRON METABOLISM OF NORMAL WOMEN [1]

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ONE FIGURE

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On the basis of iron balance studies completed before 1907, Sherman ('07) proposed our present dietary standard of an average of 15 mg. of iron daily for the normal adult. The subjects of these balance studies were men who excreted an amount of iron ranging from 7 to 16 mg. when fasting or confined to relatively restricted dietaries, such as bread and milk or bread and egg white. Since 1907, more satisfactory methods for the analysis of iron have been developed and a large amount of data is now available concerning the iron content of common foods and the influence of manufacture, preservation and cookery. When considering the sources of iron in the usual mixed dietary, these gains and losses of iron in the preparation of food and certain trends in food selection may be significant in determining the adequacy of the diet both from the standpoint of the total iron ingested and the ability of the gastro-intestinal tract to utilize the material provided. Of the iron rich foods, meat, eggs, nuts and whole grain cereals are also protein rich foods. With the present trend to reduce the amount of meat and whole grain cereals ingested (Bogert,

[1] A complete record of diets, experimental method and data on balances is on file at the library of the University of Iowa, Iowa City, Iowa.

[2] A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Department of Nutrition in the Graduate College of the State University of Iowa, June, 1934.

'31), green vegetables have been suggested as a means of balancing the iron intake. While such a balance is theoretically possible, the absolute value of green vegetables to the human organism is yet to be established. The recent review of Schultz et al. ('33) makes further discussion of this point unnecessary, except to emphasize the fact that the addition of spinach to the formula of one of his infants caused an iron excretion in excess of that supplied by the vegetable. In her study of the food intake of pregnancy, Coons ('32) reported that the least satisfactory iron retentions were found in the one subject whose diet included the greatest amount of vegetables and commented on an increased dry weight of stool during one particularly unfavorable period. Sherman ('07) suggested that slight gastro-intestinal variations might influence markedly the excretion of iron, and we find in the clinical literature (Alvarez, '31) the warning that the individual tolerance for vegetables by the human gastro-intestinal tract may be quite variable.

Of recent iron balance studies on normal individuals, the papers of Schultz, Morse and Oldham ('33), Rose et al ('30) and of Leichsenring and Flor ('32) deal with the iron exchange of infants and preschool children under the carefully controlled food conditions characteristic of those years. Reznikoff et al. ('34) have reported the iron exchange of a normal man during 7 months of hospitalization. In this time, their subject was repeatedly in iron balance on an intake of 10 mg. However, the increase of dietary iron to 26 mg. and the use of parenteral liver therapy and later of inorganic iron resulted in a large total retention and a 25 per cent increase in hemoglobin during the 7 months of observation.

This study of the iron exchange of three normal women under essentially unrestricted conditions of living and diet was undertaken since; so far as the writer is aware, no such study has been reported recently.

## EXPERIMENTAL

Only those factors which have a particular bearing upon the interpretation of the experimental data are mentioned.

The subjects studied were three healthy young women who were carrying an active schedule of laboratory work. The metabolic periods were carried out at intervals during a total of 2½ months. Periods during which complete collections were made ranged from 5 to 15 days and included at least one menstrual period for each subject. Menstruation was regular and normal in all cases, the periods of 4 to 5 days' duration at 28- to 30-day intervals, and the flow was average in amount. A total of 56 experimental days was recorded.

Each subject was consulted concerning her food likes and the approximate amount of food which she customarily ate. This information was translated into a foundation dietary which was varied in the choice of meat, vegetables and desserts. Calories were adjusted from time to time by slight variations in the bread, butter and sugar consumed. An amount of food needed for 2 or 3 days was secured at one time and stored under uniform conditions. Meals were prepared in a small kitchen resembling a household unit in its equipment of utensils. At the time the meal was served, an equivalent portion of each food was set aside for analysis and was carefully stored in Pyrex glassware. All subjects drank measured amounts of tap water from the same source and an equivalent portion was added to the total food sample. The protein and caloric adequacy of the diets was checked by means of nitrogen balances and frequent records of body weight.

All collections of food and excreta were made directly into glass containers which were covered with glass plates and were prepared for analysis without transfer. Analyzed carmen was used for marking the beginning and end of each period. During the menstrual periods, a standard brand of cellulose pad was used and a sufficient supply for the entire study was purchased in a single lot and sampled at random for blank analysis.

Sulfuric acid digests of food and feces were prepared by the method of Stearns ('29). All mineral analyses of excreta were carried out on a sulfuric acid ash prepared in 6-inch

Pyrex test tubes on a sand bath. The modification of the permanganate titration method described by Murray ('24) was adopted for the measurement of iron. A sample containing about 0.15 mg. of iron was used for analysis except in the case of urine. Copper was determined by the method of Ansbacher, Remington and Culp ('31).

TABLE 1
*Recovery data*

| MATERIAL | AMOUNT OF SAMPLE | ADDED | IN SAMPLE | FOUND | PER CENT RECOVERY |
|---|---|---|---|---|---|
| | | Iron | | | |
| | *cc.* | *mg.* | *mg.* | *mg.* | |
| Urine | 25 | 0.25 | 0.014 | 0.244 | 92.0 |
| | 15 | 0.25 | 0.002 | 0.258 | 102.4 |
| | 10 | 0.25 | 0.005 | 0.245 | 96.0 |
| | 10 | 0.25 | 0.007 | 0.249 | 96.8 |
| Feces digest | ½ | 0.25 | 0.120 | 0.364 | 97.6 |
| | ½ | 0.25 | 0.148 | 0.385 | 94.8 |
| | ½ | 0.25 | 0.108 | 0.346 | 95.2 |
| | ½ | 0.25 | 0.111 | 0.367 | 102.4 |
| Digest of menstrual pads | 1 | 0.25 | 0.113 | 0.358 | 98.0 |
| | | Copper | | | |
| Feces digest | 1 | 0.003 | 0.006 | 0.009 | 100.0 |
| | 1 | 0.01 | 0.018 | 0.027 | 90.0 |
| Food digest | 5 | 0.01 | 0.006 | 0.015 | 90.0 |
| | 5 | 0.01 | 0.006 | 0.015 | 90.0 |

## DISCUSSION OF EXPERIMENTAL RESULTS

The difficulties involved in the micro-analysis of minerals need no comment. The range of error of the iron method used was from 0 to 14 per cent with an average of 5.6 per cent. It was possible to check the copper content of duplicate samples of ash to within 13 per cent with an average of 5 per cent. Recovery data for both copper and iron are given in table 1.

Owing to the large amount of free fat present, two food samples of period 1 were lost because of 'bumping' of the wet digest. These diets were calculated from standard food tables (Waller, '31, and Hodges and Peterson, '31). The

comparison of determined and similarly calculated values for all dietaries indicated that the error introduced by this procedure would be within 10 per cent.

The menus chosen are summarized in table 2. The food ingested was generous in amount and variety and, it is be-

TABLE 2

*Summary of diets*

| PERIOD | DIET | TOTAL FOOD PORTIONS IN GRAMS | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Milk, cream, ice-cream | Butter | White bread | Meat | Potatoes | Citrous fruit[1] | Other fruit | Tomatoes[2] | Vegetables | Candy, sugar | Sandwich spread[3] | Pastry |
| 1 | 1 | 975 | 45 | 100 | 110 | 100 | 165 | 75 | 250 | 190 | 70 | 24 | 0 |
| | 2 | 950 | 45 | 100 | 110 | 100 | 165 | 80 | 250 | 130 | 60 | 37 | 0 |
| | 3 | 950 | 45 | 70 | 150 | 100 | 275 | 0 | 250 | 173 | 40 | 26 | 0 |
| | 4 | 980 | 30 | 70 | 150 | 100 | 265 | 70 | 250 | 125 | 70 | 30 | 0 |
| | 5 | 980 | 40 | 80 | 160 | 100 | 165 | 180 | 250 | 105 | 45 | 40 | 0 |
| 2 | 1 | 925 | 40 | 100 | 00[4] | 100 | 265 | 30 | 250 | 125 | 45 | 140 | 0 |
| | 2 | 945 | 40 | 100 | 45[5] | 100 | 245 | 30 | 250 | 125 | 50 | 40 | 0 |
| | 3 | 945 | 45 | 100 | 115 | 100 | 245 | 30 | 250 | 125 | 50 | 40 | 0 |
| | 4 | 915 | 45 | 100 | 140 | 100 | 265 | 30 | 250 | 125 | 45 | 40 | 0 |
| 3 | 1 | 975 | 55 | 100 | 90 | 100 | 175 | 100 | 225 | 220 | 75 | 15 | 55 |
| | 2 | 975 | 55 | 100 | 100 | 100 | 175 | 100 | 250 | 210 | 75 | 15 | 45 |
| | 3[6] | 975 | 55 | 100 | 120 | 100 | 175 | 100 | 250 | 210 | 80 | 15 | 25 |
| | 4[6] | 975 | 40 | 70 | 120 | 100 | 175 | 100 | 250 | 150 | 105 | 15 | 100 |
| | 5[6] | 975 | 40 | 70 | 120 | 100 | 175 | 100 | 250 | 210 | 120 | 15 | 45 |

[1] Includes a daily portion of 150 cc. of orange juice.

[2] Tomato juice, except in the third period when 100 gm. of fresh tomatoes were substituted for an equivalent amount of juice.

[3] Choice of egg, bacon, peanut butter, etc.

[4] Eggs were substituted.

[5] Cold roast meat.

[6] Subject C was served 0.9 of each food.

lieved, characteristic of the food habits of these subjects. Certain trends in food selection were noted: there was no cereal used, except as white bread and pastry; at least four glasses of milk were taken daily and ice cream was the choice for dessert; except in one diet where eggs were substituted, meat was used daily; sugar, candy and preserves were eaten

freely; daily portions of at least two servings of fruit and two servings of green vegetable in addition to potatoes and tomato juice were ingested. The green vegetable allowance was further increased during the third period by the addition of 100 gm. of freshly cooked green beans to each diet, except the fourth. The range of iron values for the total diets was from 11.26 to 15.57 mg. with an average of 13.78 mg. Milk furnished 14 to 16 per cent of the dietary iron, meat about 35 per cent and vegetables from 30 to 35 per cent.

The agreement between the calculated and determined values for food iron was of interest, since the method of collection of the food and the use of tap water for drinking approximated the procedure of preparing the average dietary. Determined values for iron of the diets as ingested were equal to or above the calculated value in three diets with an average increase of 3 per cent, and below the calculated values in eleven diets with an average decrease of 9 per cent. Rose et al. ('30), and Leichsenring and Flor ('32) found only 70 per cent of the calculated amounts of iron in their dietaries, but they controlled all contamination during preparation and used only distilled water for drinking purposes. From these figures, it would seem that dietary estimates of the iron intake based on the data of available food tables would yield satisfactory results for meal planning in any situation other than the strict metabolic study.

Complete daily and period balances are summarized in table 3. In studying the iron excretions, only the fecal and menstrual iron losses were significant in determining the iron balance, although iron was found in all except one of the urine samples. The average daily output of iron in all urines was 0.52 mg. When the menstrual specimens which contained blood were excluded, an average figure of 0.38 mg. was obtained. Subject A seemed to show a tendency to excrete greater amounts of urinary iron than subjects B and C. Coons ('32) commented on a similar individual trend in certain of of her cases.

Since fecal iron was determined daily, the curve of excretion was very irregular. However, a parallelism of fetal nitrogen and fetal iron was noted particularly on the days of maximum and minimum excretion (fig. 1). Coons ('32) and Reznikoff ('34) observed a relationship between iron output and dry weight of stool. The total fecal losses of iron, however, were excessive when slight gastro-intestinal discomfort and increased elimination followed the ingestion of string beans (subject A).

Subject A was in positive iron balance during the first two periods, but lost 51 mg. of iron during the third and longest period in which she was studied. During the 6 days of period 1, this subject stored 14 mg. of iron. This amount was equal to about 75 per cent of the 18 mg. lost in menstruation, which occurred on the first 4 days of the period. In period 2, which represented the inter-menstrual phase of the same monthly cycle, she was still retaining an average of 0.44 mg. of iron daily. Period 3 shows an average daily loss of iron of 3.43 mg. There are two factors to be considered in interpreting this finding: first, menstruation which occurred from the second to the fifth day, inclusive, and, second, the addition of an extra portion of fresh green vegetable to the diet for the first 9 and the last 3 days of the period. An average daily increase of fecal nitrogen of 0.17 gm. occurred in this period (fig. 1). After the first few days, the subject complained of gas and general discomfort and attributed the difficulty to the added vegetable. The green beans were discontinued for 3 days and the fecal nitrogen fell on the third day to below the level of periods 1 and 2, but rose when the vegetable was included again. For the first 5 days of this period, which included menstruation, the average iron balance was positive with a daily average storage of 3.61 mg. However, from this point the fecal iron rose, reaching a peak of 32.48 mg. on the eighth day, and 29.79 and 28.37 mg. on the tenth and eleventh days, respectively. The average daily amount ingested was 13.76 mg. After discontinuing the string beans, a positive balance averaging 1.98 mg. was found for the twelfth, thir-

TABLE 3

*Summary of balance studies*

| SUBJECT | PERIOD | DIET | NITROGEN | | | IRON | | | COPPER | | | COMMENT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Intake | Output | Balance | Intake | Output | Balance | Intake | Output | Balance | |
| | | | *gm.* | *gm.* | | *mg.* | *mg.* | | *mg.* | *mg.* | | |
| A | 1 | 1 | 11.99[1] | 10.77 | + 1.22 | 14.41[1] | 1.29 | + 13.12 | 0.97[1] | 0.00 | + 0.97 | Menstruation (some material lost on clothing) |
| | | 1 | 11.99[1] | 12.77 | — 0.78 | 14.41[1] | 21.38 | — 6.97 | 0.97[1] | 2.90 | — 1.73 | |
| | | 2 | 13.10 | 10.71 | + 2.49 | 13.64 | 1.06 | + 12.58 | 0.89 | 0.00 | + 0.89 | |
| | | 2 | 13.10 | 13.10 | 0.00 | 13.64 | 23.90 | — 10.26 | 0.89 | 2.60 | — 1.71 | |
| | | 3 | 12.72[1] | 10.90 | + 1.82 | 13.66[1] | 1.25 | + 12.41 | 1.03[1] | 0.00 | + 1.03 | |
| | | 3 | 12.72[1] | 10.41 | + 2.31 | 13.66[1] | 20.20 | — 6.54 | 1.03[1] | 2.69 | — 1.66 | |
| | Averages | | | | | | | | | | | |
| | Entire period | | 12.60 | 11.44 | + 1.16 | 13.90 | 11.51 | + 2.39 | 0.96 | 1.37 | — 0.40 | |
| | Menstruation 4 days | | 12.55 | 11.84 | + 0.71 | 14.03 | 11.97 | + 2.12 | 0.93 | 1.38 | — 0.45 | |
| | Post menstruation 2 days | | 12.72 | 10.66 | + 2.06 | 13.66 | 10.73 | + 2.94 | 1.03 | 1.35 | — 0.22 | |
| A | 2 | 2 | 10.81 | 11.26 | — 0.45 | 11.26 | 22.17 | — 10.91 | 0.93 | 2.09 | — 1.16 | |
| | | 2 | 10.81 | 11.84 | — 1.03 | 11.26 | 6.84 | + 4.42 | 0.93 | 0.86 | + 0.07 | |
| | | 3 | 12.17 | 12.31 | — 0.14 | 13.90 | 19.77 | — 5.87 | 1.12 | 1.49 | — 0.37 | |
| | | 3 | 12.17 | 13.39 | — 1.22 | 13.90 | 8.73 | + 5.17 | 1.12 | 0.93 | + 0.19 | |
| | | 3 | 12.17 | 10.82 | + 1.35 | 13.90 | 4.49 | + 9.41 | 1.12 | 0.75 | + 0.37 | |
| | Averages | | | | | | | | | | | |
| | Inter mestruation. Total | | 11.63 | 11.92 | — 0.29 | 12.84 | 12.40 | + 0.44 | 1.04 | 1.22 | — 0.18 | |
| | Diet 2, 2 days | | 10.81 | 11.55 | — 0.74 | 11.26 | 14.51 | — 3.25 | 0.93 | 1.88 | — 0.55 | |
| | Diet 3, 3 days | | 12.17 | 12.17 | 0.00 | 13.90 | 11.00 | + 2.90 | 1.12 | 1.06 | + 0.06 | |
| A | 3 | 1 | 11.05 | 8.82 | + 2.23 | 13.54 | 0.24 | + 13.30 | 1.16 | 0.00 | + 1.16 | Menstruation |
| | | 1 | 11.05 | 10.20 | + 0.85 | 13.54 | 20.82 | — 7.28 | 1.16 | 0.91 | — 0.75 | |
| | | 1 | 11.05 | 10.29 | + 0.76 | 13.54 | 22.45 | — 8.91 | 1.16 | 1.80 | — 0.64 | |
| | | 2 | 10.98 | 9.65 | + 1.33 | 15.36 | 9.52 | + 5.84 | 1.35 | 1.06 | + 0.29 | |
| | | 2 | 10.98 | 9.16 | + 1.82 | 15.36 | 0.27 | + 15.09 | 1.35 | 0.00 | + 1.35 | |
| | | 2 | 10.98 | 12.92 | — 1.94 | 15.36 | 28.17 | — 12.81 | 1.35 | 2.14 | — 0.79 | |
| | | 3 | 11.63 | 10.25 | + 1.38 | 13.74 | 9.23 | + 4.51 | 1.11 | 0.93 | + 0.18 | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 10.98 | 9.16 | + 1.82 | 15.36 | 9.27 | + 6.09 | 1.35 | 1.67 | — 0.32 | |
| | | | 2 | 10.98 | 12.92 | — 1.94 | 15.36 | 28.17 | — 12.81 | 1.35 | 2.14 | — 0.79 | |
| | | | 3 | 11.63 | 10.25 | + 1.38 | 13.74 | 9.23 | + 4.51 | 1.11 | 0.93 | + 0.18 | |
| | | | 3 | 11.63 | 12.69 | — 1.06 | 13.74 | 32.48 | — 18.74 | 1.11 | 1.67 | — 0.56 | |
| | | | 3 | 11.63 | 10.27 | + 1.36 | 13.74 | 17.83 | — 4.09 | 1.11 | 1.68 | — 0.57 | |
| | | | 4 | 11.00 | 11.33 | — 0.33 | 12.49 | 29.79 | — 17.30 | 1.32 | 1.43 | — 0.11 | |
| | | | 4 | 11.00 | 10.10 | + 0.90 | 12.49 | 28.37 | — 16.07 | 1.32 | 0.84 | + 0.48 | |
| | | | 4 | 11.00 | 10.40 | + 0.60 | 12.49 | 18.40 | — 5.91 | 1.32 | 1.46 | — 0.14 | |
| | | | 5 | 10.83 | 9.10 | + 1.73 | 13.65 | 0.31 | + 13.34 | 0.80 | 0.00 | + 0.80 | |
| | | | 5 | 10.83 | 11.55 | — 0.72 | 13.65 | 15.13 | — 1.48 | 0.80 | 1.85 | — 1.05 | |
| | | | 5 | 10.83 | 10.80 | + 0.03 | 13.65 | 24.53 | — 10.88 | 0.80 | 1.63 | — 0.83 | |
| | | | | | | | **Averages** | | | | | | |
| | **Total** | | 11.10 | 10.50 | + 0.60 | 13.76 | 17.18 | — 3.43 | 1.15 | 1.23 | — 0.08 | |
| | **Pre-menstruation 1 day** | | 11.05 | 8.82 | + 2.23 | 13.54 | 0.24 | + 13.30 | 1.16 | 0.00 | + 1.16 | |
| | **Menstruation 4 days** | | 11.02 | 9.83 | + 1.19 | 14.45 | 13.27 | + 1.19 | 1.26 | 1.19 | + 0.07 | |
| | **Post menstruation 4 days** | | 11.47 | 11.53 | — 0.06 | 14.15 | 21.13 | — 7.78 | 1.17 | 1.61 | — 0.44 | |
| | **3 days** | | 11.00 | 10.61 | + 0.39 | 12.49 | 25.58 | — 13.09 | 1.32 | 1.24 | + 0.08 | |
| | **3 days** | | 10.83 | 10.48 | + 0.35 | 13.65 | 13.32 | + 0.33 | 0.80 | 1.16 | — 0.36 | |
| **B** | **2** | **1** | 10.05 | 11.26 | — 1.21 | 12.62 | 20.93 | — 8.31 | 0.86 | 1.87 | — 1.01 | **Menstruation** |
| | | **1** | 10.05 | 9.05 | + 1.00 | 12.62 | 17.96 | — 5.34 | 0.86 | 0.61 | + 0.25 | |
| | | **1** | 10.05 | 9.42 | + 0.63 | 12.62 | 18.97 | — 6.35 | 0.86 | 0.92 | — 0.06 | |
| | | **2** | 10.81 | 9.42 | + 1.39 | 11.26 | 13.03 | — 1.77 | 0.93 | 1.32 | — 0.39 | |
| | | **2** | 10.81 | 10.54 | + 0.27 | 11.26 | 19.31 | — 8.05 | 0.93 | 1.23 | — 0.30 | |
| | | **2** | 10.81 | 12.55 | — 1.74 | 11.26 | 19.47 | — 8.21 | 0.93 | 1.20 | — 0.27 | |
| | | **3** | 12.17 | 11.60 | + 0.57 | 13.90 | 8.09 | + 5.81 | 1.12 | 0.66 | + 0.46 | |
| | | **3** | 12.17 | 11.63 | + 0.54 | 13.90 | 8.23 | + 5.67 | 1.12 | 1.40 | — 0.28 | |
| | | **3** | 12.17 | 12.41 | — 0.24 | 13.90 | 20.32 | — 6.42 | 1.12 | 1.78 | — 0.66 | |
| | | **4** | 12.04 | 11.45 | + 0.59 | 15.57 | 14.26 | + 1.31 | 1.12 | 0.48 | + 0.64 | |
| | | **4** | 12.04 | 12.94 | — 0.90 | 15.57 | 12.03 | + 3.54[3] | 1.12 | 1.20 | — 0.08 | |
| | | **4** | 12.04 | 11.95 | + 0.09 | 15.57 | 7.04 | + 8.53 | 1.12 | 0.84 | + 0.28 | |
| | | | | | | | **Averages** | | | | | |
| | **Total 12 days** | | 11.27 | 11.16 | + 0.11 | 13.34 | 15.00 | — 1.67 | 1.01 | 1.13 | — 0.12 | |
| | **Pre-menstruation 1 day** | | 10.05 | 11.26 | — 1.21 | 12.62 | 20.93 | — 8.31 | 0.86 | 1.87 | — 1.01 | |
| | **Menstruation 4 days** | | 10.43 | 9.61 | + 0.82 | 11.94 | 17.42 | — 5.48 | 0.90 | 1.02 | — 0.12 | |
| | **Post menstruation 4 days** | | 11.83 | 12.02 | — 0.19 | 13.24 | 14.03 | — 0.79 | 1.07 | 1.26 | —.0.19 | |
| | **3 days** | | 12.04 | 12.11 | — 0.07 | 15.57 | 11.11 | + 4.46 | 1.12 | 0.84 | + 0.28 | |

TABLE 3—*Continued*

| SUBJECT | PERIOD | DIET | NITROGEN | | | IRON | | | COPPER | | | COMMENT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Intake | Output | Balance | Intake | Output | Balance | Intake | Output | Balance | |
| | | | *gm.* | *gm.* | | *mg.* | *mg.* | | *mg.* | *mg.* | | |
| C | 1 | 2 | 13.10 | 11.18 | + 1.92 | 13.64 | 18.56 | — 4.92 | | | | Menstruation |
| | | 3 | 12.72[1] | 10.39 | + 2.33 | 13.66[1] | 12.19 | + 1.47 | | | | |
| | | 3 | 12.72[1] | 9.98 | + 2.74 | 13.66[1] | 12.08 | + 1.58 | | | | |
| | | 4 | 11.96 | 12.55 | — 0.59 | 13.60 | 13.27 | + 0.33 | | | | |
| | | 4 | 11.96 | 12.13 | — 0.17 | 13.60 | 16.01 | — 2.41 | | | | |
| | | 5 | 13.40 | 13.83 | — 0.43 | 15.47 | 17.70 | — 2.22 | | | | |
| | | 5 | 13.40 | 13.29 | + 0.11 | 15.47 | 15.75 | — 0.27 | | | | |
| | Averages | | | | | | | | | | | |
| | Total | | 12.75 | 11.91 | + 0.84 | 14.16 | 15.08 | — 0.90 | | | | |
| | Menstruation 5 days | | 12.49 | 11.13 | + 1.36 | 13.63 | 14.42 | — 0.59 | | | | |
| | Post menstruation | | 13.40 | 13.56 | — 0.16 | 15.48 | 16.73 | — 1.25 | | | | |
| C | 3 | 2 | 10.98 | 10.02 | + 0.96 | 15.36 | 19.55 | — 4.19 | 1.35 | 1.09 | + 0.24 | |
| | | 2 | 10.98 | 11.05 | —.0.07 | 15.36 | 11.90 | + 3.46 | 1.35 | 1.23 | + 0.12 | |
| | | 3[2] | 10.47 | 7.92 | + 2.55 | 12.37 | 15.16 | — 2.79 | 1.11 | 0.79 | + 0.32 | |
| | | 3 | 10.47 | 10.04 | + 0.43 | 12.37 | 10.53 | + 1.84 | 1.11 | 0.84 | + 0.27 | |
| | | 3 | 10.47 | 9.61 | + 0.86 | 12.37 | 13.27 | — 0.90 | 1.11 | 0.98 | + 0.15 | |
| | | 4[2] | 9.90 | 9.79 | + 0.11 | 11.24 | 19.60 | — 8.36 | 1.32 | 2.27 | — 0.95 | |
| | | 4 | 9.90 | 8.69 | + 1.21 | 11.24 | 10.67 | + 0.57 | 1.32 | 0.98 | — 0.34 | |
| | | 4 | 9.90 | 7.86 | + 2.04 | 11.24 | 11.64 | — 0.40 | 1.32 | 0.59 | + 0.73 | |
| | | 5[2] | 9.75 | 9.07 | + 0.68 | 12.28 | 12.65 | — 0.37 | 0.80 | 1.50 | + 0.70 | |
| | | 5 | 9.75 | 9.58 | + 0.17 | 12.28 | 22.52 | — 10.24 | 0.80 | 1.30 | — 0.50 | |
| | | 5 | 9.75 | 10.28 | — 0.53 | 12.28 | 16.02 | — 3.74 | 0.80 | 1.06 | — 0.26 | |
| | Averages | | | | | | | | | | | |
| | Total | | 10.21 | 9.45 | +0.76 | 12.58 | 14.87 | — 2.29 | 1.13 | 1.14 | — 0.02 | |
| | Dietary Periods 1, 2 days | | 10.98 | 10.54 | + 0.44 | 15.36 | 15.73 | — 0.37 | 1.35 | 1.16 | + 0.19 | |
| | 2, 3 days | | 10.47 | 9.19 | + 1.28 | 12.37 | 12.99 | — 0.62 | 1.11 | 0.87 | + 0.24 | |
| | 3, 3 days | | 9.90 | 8.78 | + 1.12 | 11.24 | 13.98 | — 2.74 | 1.32 | 1.28 | +0.04 | |
| | 4, 3 days | | 9.75 | 9.64 | + 0.11 | 12.28 | 17.06 | — 4.78 | 0.80 | 1.29 | — 0.49 | |

[1] Calculated figures.

[2] Urine figure omitted because of contaminated specimen.

[illegible] Subject A requested specific diet of the dietary as omitted

teenth and fourteenth days, but the effect of including the vegetable again was evident on the last day when the retention fell to a negative 10.88 mg.

Subject C lost a total daily average of 2.29 mg. of iron during the third period. This subject was in slightly negative iron balance during the first period also. In addition to the 25.68 mg. of iron lost in menstruation, her total urine and fecal output averaged 15.08 mg. on a daily intake of 14.16 mg.

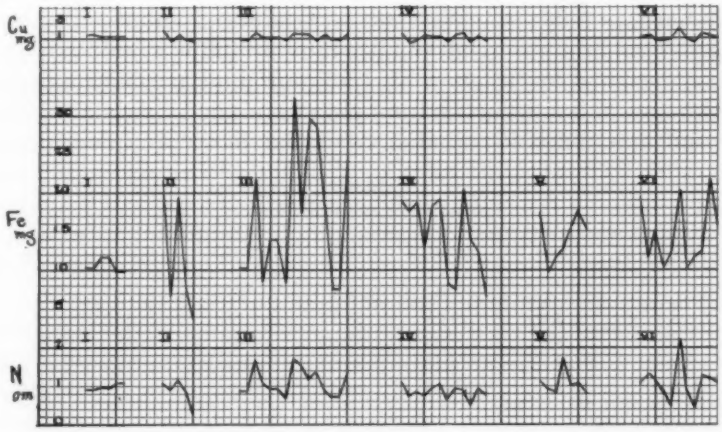


Fig. 1 Comparison of fecal copper, iron and nitrogen.

I, Subject A, period 1
II, Subject A, period 2
III, Subject A, period 3
IV, Subject B, period 2
I, Subject C, period 1
VI, Subject C, period 3

This subject had had the least experience in metabolic studies and just what part emotional adjustment played in determining her gastro-intestinal efficiency is speculative.

The findings on subject B during the single period of observation offer an interesting contrast to the periods for subject A. Subject B was in strikingly negative iron balance during menstruation since she lost an average of 6.34 mg. of iron for each of 6 days. The next 3-day period was marked by a positive balance of 1.35 mg. per day and this balance in

[1] Calculated figures.
[2] Urine figure omitted because of contaminated specimen.

the last 3 days of the period increased to an average of 4.46 mg. The parallelism between fecal nitrogen and fecal iron was again striking. Although the total iron balance of this subject for the period was negative to the extent of a daily loss of 1.67 mg., the rate of storage during the last 6 days indicated that rapid compensation was taking place. This subject lost 41.90 mg. of iron in the menstrual hemorrhage which is the largest amount recorded in this series.

The complete data for the losses of menstruation are summarized in table 4. With the exception of subject B, the menstrual losses were all smaller than the 34, 38 and 42 mg. of iron reported by Gillett et al. ('18) for three normal women. Although these reported iron losses are not large, they will result in a deficit if repeated at regular intervals unless preceded or followed by a storage of iron. Such a deficit is suggested by the hypochromic anemia of women described by Bloomfield ('32), who found a history of excessive blood losses in only a small proportion of cases. Mettier ('31) reported a series of hypochromic anemia cases where a deficient dietary consumed over a period of years appeared to be an etiological factor. The nitrogen losses of the menstrual hemorrhage were also low as compared with the range of from 0.656 to 1.92 gm. found by Sherman, Gillett and Pope ('18).

TABLE 4
*Analysis of menstrual loss*

| SUBJECT | PERIOD | NITROGEN | IRON | COPPER | FE : N | CU : FE |
|---|---|---|---|---|---|---|
| | | *gm.* | *mg.* | *mg.* | | |
| A | 1 | 0.64 | 18.17 | 0.39 | 1 : 35 | 1 : 46 |
| A | 3 | 0.89 | 32.35 | 0.61 | 1 : 28 | 1 : 53 |
| B | 2 | 0.46 | 41.92 | 0.19 | 1 : 11 | 1 : 221 |
| C | 1 | 0.80 | 25.68 | 0.24 | 1 : 31 | 1 : 107 |

Dietary copper as determined ranged from 0.80 to 1.39 mg. per day. The calculated copper value for the ten diets ranged from 6 to 37 per cent higher than the determined values. The copper to iron ratio of the dietaries was from 1 to 12 to 1 to 17.

Copper was found in traces in twenty-four out of twenty-seven urine samples which were analyzed, but the amount was in no case sufficient for calculation. This was somewhat amazing in view of the findings of Rabinowitch ('33), who reported a daily urinary copper ranging from a trace to 0.70 mg. An examination of the copper in the stool, however, indicated that the amounts ingested could be found in the fecal output. Negative copper balances ranging from a daily average of 0.02 to 0.40 mg. were observed. In all cases, except subject A in periods 1 and 2, the average amount of copper lost was within 10 per cent of the intake, which is perhaps also within the limitations of the method. In period 1, subject A lost a daily average of 0.40 mg. and in period 2 a daily negative retention of 0.18 mg. was observed. The subject was storing iron during both of these periods. There appeared to be some tendency for high excretions of copper to parallel high fecal iron, but this parallelism was not entirely consistent.

It would seem unwise to generalize from the few observations presented here. There seemed to be an extremely labile mechanism regulating the iron balance and that this mechanism was closely related to intestinal function was obvious. These normal subjects were apparently able to compensate for temporary intestinal inefficiency with marked iron retentions when the irritating situation was relieved. It would be reasonable to suppose, however, that repeated gastro-intestinal irregularities might exhaust the iron reserves, particularly if the intake of food were limited. Although the average iron content of these diets did not quite reach the amount suggested by Sherman ('07) as an optimal intake, the diets as a whole were generous in all known food constituents. Sherman ('07) has correlated a lack of calcium with negative iron balances. The calcium intake of this study was in excess of a gram per day. The part which vegetable fiber played in determining the response to string beans is open to conjecture. Adolph and Wu ('34) did not find an increased acceleration of the passage of food through the tract or an increase in fecal

nitrogen when cabbage fiber was fed at high levels. If fruit and fruit juices are included in the total, the vegetable intake of all the diets used in this study was between 500 and 700 gm., but almost half of the amount was in the form of strained fruit juices so that the fiber content of the diets was not raised proportionately.

## SUMMARY

1. Iron balances are presented on three normal women for six periods totaling 56 experimental days. On self-chosen diets containing liberal amounts of protein, calories and protective elements, the average daily amount of iron ingested was 13.78 mg. The average daily iron excretion for all periods was 14.95 mg.

2. Two periods of positive iron balance were recorded for subject A. Subjects B and C were both in negative iron balance as was subject A for the third period of study. Negative iron balances were associated with increased fecal nitrogen. No relationship was observed between iron retention and the iron losses of menstruation or between the balance of nitrogen or copper and the balance of iron.

3. Totals of 18.16, 25.68, 32.35 and 41.90 mg. of iron, respectively, were lost in the menstrual hemorrhage during each of four periods.

4. The copper to iron ratios of the ingested food ranged from 1 to 12 to 1 to 17. Copper was excreted through the stool.

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# A STUDY OF THE PROTEIN NEEDS OF PRESCHOOL CHILDREN

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THREE CHARTS

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There is little information in the literature relative to the amount of protein which children of various ages beyond infancy should be given. From 10 to 12 per cent of the total calories, or approximately 1 gm. per pound of body weight is considered sufficient by most students of child nutrition, "provided the diet is of ample fuel value and the protein is of the right kind" (Sherman, '18). These standards have been arrived at more or less empirically, the results of observations of growth when the children were receiving 1 gm. or thereabouts per pound, and by a consideration of the percentage of the protein of human milk—a food presumably adapted to the needs of the child during its most rapidly growing period. "Practically, it is not possible to keep the protein as low as 10 per cent of the total calories and still observe the rule of a quart of milk per day. There seems to be no reason why conditions for growth need be less favorable when the protein calories constitute as much as 15 per cent of the total calories" (Rose, '27). Holt ('21) reports in a study of the food eaten by thirty-two children from 2 to 6 years of age in a children's home that an average of 3.5 gm. of protein per kilogram was taken daily; and an estimate of the protein eaten by normal healthy children of intelligent well-to-do families showed that the children be-

tween 2 and 3 years of age were taking 49 gm. or approximately 4 gm. per kilogram. Older children between 6 and 7 averaged 60 gm., or 3 gm. per kilogram. These data do not necessarily indicate child needs, but rather the amount contained in the foods eaten.

The most extensive study of the nitrogen metabolism of children is that of Wang, Hawks and Hays ('28) with both normal and underweight children between 5 and 13 years of age. Based on weights and observations of the activity of the children, these authors have grouped their findings into several categories: nitrogen metabolism of vigorous normal children; of children normal in weight only; of children from 5 to 10 per cent underweight, etc. The average nitrogen retentions of the various groups conformed rather consistently to these groupings, less being retained by the vigorous normal group and larger amounts by those who were below weight. For example, the average retention of the vigorous normal group reported was 0.041 gm. at an average ingestion of 0.448 gm. per kilogram, whereas the children who were normal in weight only retained an average of 0.088 gm. per kilogram at an ingestion of 0.516 gm. per kilogram. As the percentage of underweight increased, the average amount ingested as well as the amount retained increased. There were, however, quite wide variations among the individuals of different groups. These may be explained possibly by differences in the ages of the children or by variations in the amount of nitrogen ingested.

In a second study (Wang, Hawks and Kaucher, '28) retentions with both high and low ingestions were determined. In this as in the former study, the underweight children retained more than those of average weight whether on high or low protein diets. At an average ingestion of 0.537 gm. per kilogram by the normal children, more nitrogen was retained (0.075 gm. per kilogram) than in the previous study at the somewhat lower ingestion level (0.448 gm. per kilogram). With a much lower ingestion, 0.264 gm. per kilogram (corresponding to 0.75 gm. of protein per pound) an average

of only 0.001 gm. per kilogram was retained. Several of the children at this low ingestion level were found to be in negative balance perhaps due to the fact that an insufficient amount of high grade protein was included in the diet.

That the protein requirement of children varies inversely with age and is directly proportional to the rate of growth is well known. In Wang's studies there was but one child below 8 years of age among the vigorous normal group (Wang, Hawks and Hays, '28). In the groups normal in weight only there are reported only five children 6 years of age or under who were up to standard weight. The nitrogen retentions of these varied from 0.026 to 0.172 gm. per kilogram on the low protein diet, whereas the retention of those slightly below average varied from 0.064 to 0.158 gm.; and although the ingestions also varied, with the exception of those on the very low ingestions, there appeared to be no significant correlation between the amount ingested and the amount retained. Altogether, there would seem to be too few cases from which to draw conclusions regarding the protein needs of the preschool child, a group just beyond babyhood which may need considerably more than the older child of whom Wang's group largely consisted.

Parsons ('30), in a series of nitrogen studies with diabetic children from 4 to 8 years of age, obtained positive balances with 0.5 gm. of protein per pound or 1.1 gm. per kilogram; similarly, Bartlett ('26), also studying diabetic children between 4 and 14 years of age, obtained positive nitrogen balances, growth in height, and gain in weight with normal activity at ingestion levels of 0.6 to 1.0 gm. of protein per kilogram. It will be noted that the amount of protein taken by these children was considerably less than that taken by the children reported by Holt, or than the generally accepted amount of 1 gm. per pound, lower even than the low levels used by Wang for children of similar ages with whom some negative balances were obtained. Are these low amounts used by Parsons and Bartlett sufficient, or are the higher amounts more nearly in accord with the child's optimum physiological

tween 2 and 3 years of age were taking 49 gm. or approximately 4 gm. per kilogram. Older children between 6 and 7 averaged 60 gm., or 3 gm. per kilogram. These data do not necessarily indicate child needs, but rather the amount contained in the foods eaten.

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performance? A positive nitrogen balance obviously cannot be the criterion by which to judge the protein needs of a given child. Growth is a prerogative of childhood. The rate of growth is limited by the inherent capacity of the individual and the available amount of any one of the various essential nutritive constituents between the minimum and the optimum; and although the children in both Parsons' and Bartlett's studies gained in weight and height, they may not have realized their inherent growth capacities. At any level of protein ingestion above that required to cover katabolism, a positive nitrogen balance would obtain, provided adequate protein were supplied. Furthermore, it seems probable that the nitrogen needs of children even at the same age differ, depending upon the inherent potentialities of growth. Not knowing this, enough protein should be given to cover possible needs. How much this is can be determined only by studies with a considerable number of children of various ages and at various levels of ingestion.

In the investigation herein reported, 121 nitrogen balance studies have been made with 24 normal children (15 boys and 9 girls) between 37 and 66 months of age. During the observation period, the children lived under controlled conditions from 14 to 21 days as described in previous reports from this laboratory (Daniels et al., '34). The caloric needs of the children were considered from the standpoint of both height and weight. With but few exceptions, food supplying 40 calories per inch, or between 80 and 112 calories per pound was given. During the study period, the children gained in weight, thus ruling out the possibility of undernutrition as a factor influencing the nitrogen retentions. The amount of protein given varied from 0.9 to 1.6 gm. per pound of body weight. This was divided evenly among the three meals. The actual amount fed necessarily differed somewhat from the estimated amount because of the fact that the food used varied slightly in composition from that of the published analyses and the children gained during the period; thus the final weights, the average of the daily weights used in calcula-

tions of ingestions and retentions, were often higher than the weights used when the diets were planned.

## EXPERIMENTAL PROCEDURE

In the first part of the study, from 3 to 4 days were allowed for metabolic adjustments to the diets before the balance studies were made. In general, two periods of 5 days each exclusive of the preliminary adjustment periods constituted a first and a second metabolism period at a given nitrogen ingestion level. Because of the differences sometimes observed in the first and second successive metabolism periods, later in the study a longer period (from 5 to 7 days) was allowed for the metabolic adjustment, and in these the metabolism periods (called double periods) were extended to 10 days, thus ruling out possible errors which might accrue from incomplete fecal eliminations of a shorter period. Stools in all periods were marked with 0.2 gm. of carmine.

A change in the method of preparing certain of the foods also was made for these longer metabolism periods, partly for the purpose of facilitating the procedures and partly for greater accuracy. With the exception of the meat and eggs, all cooked foods were prepared at the beginning of the metabolism period, subsequently packed in sterile containers, and stored in a low temperature refrigerator. Meat for each serving was weighed at the beginning of the period, wrapped in waxed paper and kept frozen until ready for use. Distilled water only was used for cooking and drinking. All children received daily from 2 to 4 ounces of orange juice and 2 teaspoons of cod liver oil. Many of them received also four drops of Viosterol twice daily. This apparently was without influence on the nitrogen retentions.

Nitrogen determinations by the Kjeldahl method were made with aliquots of each of the 24-hour urinary excretions, whereas aliquots of the total dried fecal excretions of the entire period and aliquots of the food for a given day were used.

In the investigation, presumably only physically fit children were studied, that is, they were active, carried no elevated temperatures, exhibited no digestive disturbances, slept well and, with very few exceptions, ate well. Physical measurement standards for children of this age group are limited, the most extensive being those of the Iowa Child Welfare Research Station ('29). Comparing the weight for height of the children studied with these standards, it was found that the children in general compared favorably with these norms. A few, however, were above the average, while others were below. One child who was studied during ten periods remained about 11 per cent below his theoretical weight for height. Twenty-five per cent of the children came from a state orphanage, 25 per cent from a county juvenile home where the food conditions were exceptionally good, and 50 per cent from private homes.

In determining nitrogen retentions in terms of weight it is obvious that if a child is underweight, a higher retention value will be obtained than if this is determined in terms of normal weight. A similar situation with regard to ingestion prevails. Therefore, both ingestions and retentions in the children studied have been determined, 1) on the basis of actual weight, and 2) on the basis of theoretical weight. These have been grouped according to ingestions on a scale of five between 0.35 and 0.70 gm. per kilogram, first, second, and double metabolism periods being grouped separately.

## RESULTS

With the exception of a few cases at the very high and low ingestion levels, higher nitrogen retentions were obtained during first metabolism periods, irrespective of the amount ingested, within the limits of the study. For example, with nitrogen ingestions between 0.55 and 0.59 gm. per kilogram, eight children who averaged slightly below (— 3.5 per cent) the normal weight for height retained 0.121 gm. per kilogram during the first metabolism periods, whereas six at the same ingestion level retained an average of 0.084 gm. during second

metabolism periods (table 1). Similarly, seventeen children very slightly above the average weight (+ 1.4 per cent), receiving between 0.50 and 0.54 gm. per kilogram, retained 0.101 during first periods, and eleven, also slightly above (+ 1.1 per cent) average weight for height, retained 0.092 gm. during second successive periods.

TABLE 1

*Relation between nitrogen ingestion and nitrogen retention based on actual weights of children*

| NITROGEN INGESTION PER KILOGRAM | NUMBER OF CASES | AVERAGE AGE | AVERAGE WEIGHT | NITROGEN RETENTION | | | PER CENT OVER + OR UNDER − AVERAGE WEIGHT | |
|---|---|---|---|---|---|---|---|---|
| | | | | Average per kilogram | Standard deviation | Range per kilogram | Average | Range |
| **First metabolism periods** | | | | | | | | |
| *gm.* | | *mo.* | *kg.* | *gm.* | *gm.* | *gm.* | | |
| 0.70–0.74 | 0 | — | — | — | — | — | — | — |
| 0.65–0.69 | 3 | 65 | 16.3 | 0.114 | — | 0.111–0.117 | — 7.9 | —11.3, — 4.1 |
| 0.60–0.64 | 4 | 63 | 16.7 | 0.112 | — | 0.100–0.141 | — 4.4 | —12.5, + 5.0 |
| 0.55–0.59 | 8 | 53 | 15.4 | 0.121 | 0.017 | 0.096–0.153 | — 3.5 | —11.1, + 5.1 |
| 0.50–0.54 | 17 | 53 | 16.2 | 0.101 | 0.022 | 0.056–0.141 | + 1.4 | — 9.8, + 9.5 |
| 0.45–0.49 | 15 | 57 | 16.9 | 0.083 | 0.021 | 0.054–0.117 | + 2.7 | — 4.9, +15.4 |
| 0.40–0.44 | 11 | 58 | 18.1 | 0.066 | 0.009 | 0.047–0.077 | + 3.5 | — 4.0, + 7.6 |
| 0.35–0.39 | 1 | 53 | 14.7 | 0.063 | — | — | — 4.6 | — |
| **Second metabolism periods** | | | | | | | | |
| 0.70–0.74 | 1 | 73 | 16.8 | 0.107 | — | — | — 7.6 | — |
| 0.65–0.69 | 1 | 60 | 15.8 | 0.143 | — | — | —11.3 | — |
| 0.60–0.64 | 2 | 61 | 17.4 | 0.101 | — | 0.083–0.122 | + 0.3 | — 5.7, + 6.2 |
| 0.55–0.59 | 6 | 57 | 15.7 | 0.084 | 0.019 | 0.061–0.120 | — 5.4 | —12.0, + 3.2 |
| 0.50–0.54 | 11 | 53 | 15.9 | 0.092 | 0.019 | 0.060–0.119 | + 1.1 | —12.3, + 9.1 |
| 0.45–0.49 | 13 | 61 | 18.0 | 0.078 | 0.02 | 0.044–0.117 | + 5.0 | — 3.3, +11.6 |
| 0.40–0.44 | 6 | 61 | 18.9 | 0.068 | — | 0.053–0.084 | + 4.7 | — 3.5, +11.5 |
| 0.35–0.39 | 0 | — | — | — | — | — | — | — |
| **Double metabolism periods** | | | | | | | | |
| 0.70–0.74 | 0 | — | — | — | — | — | — | — |
| 0.65–0.69 | 1 | 54 | 15.9 | 0.099 | — | — | — 4.9 | — |
| 0.60–0.64 | 3 | 61 | 16.9 | 0.097 | — | 0.091–0.110 | — 4.3 | — 7.9, + 2.6 |
| 0.55–0.59 | 7 | 51 | 16.5 | 0.075 | 0.013 | 0.054–0.092 | — 0.7 | — 8.0, + 8.5 |
| 0.50–0.54 | 2 | 61 | 16.8 | 0.086 | — | 0.077–0.095 | — 1.3 | — 4.1, + 1.5 |
| 0.45–0.49 | 0 | — | — | — | — | — | — | — |
| 0.40–0.44 | 8 | 62 | 17.1 | 0.069 | 0.013 | 0.052–0.096 | + 2.2 | — 5.2, +16.0 |
| 0.35–0.39 | 1 | 40 | 14.4 | 0.055 | — | — | — 0.4 | — |

With the exception of two cases at ingestions between 0.060 and 0.064 gm., retentions estimated on the theoretical weights showed similar relationships at the higher levels, that is, higher retentions during first metabolism periods, but somewhat closer agreement at the lower levels (table 2). With ingestions between 0.45 and 0.49 gm. per kilogram, the

TABLE 2

*Relation between nitrogen ingestion and nitrogen retention based on theoretical weights of children*

| NITROGEN INGESTION PER KILOGRAM | NUMBER OF CASES | AVERAGE AGE | AVERAGE WEIGHT | NITROGEN RETENTION | | | PER CENT OVER + OR UNDER − AVERAGE WEIGHT | |
|---|---|---|---|---|---|---|---|---|
| | | | | Average per kilogram | Standard deviation | Range per kilogram | Average | Range |
| **First metabolism periods** | | | | | | | | |
| *gm.* | | *mo.* | *kg.* | *gm.* | *gm.* | *gm.* | | |
| 0.70–0.74 | 0 | — | — | — | — | — | — | — |
| 0.65–0.69 | 0 | — | — | — | — | — | — | — |
| 0.60–0.64 | 4 | 61 | 16.7 | 0.114 | — | 0.105–0.133 | − 0.8 | − 8.4, + 5.0 |
| 0.55–0.59 | 13 | 57 | 17.0 | 0.114 | 0.027 | 0.068–0.152 | + 2.4 | −11.3, +12.5 |
| 0.50–0.54 | 16 | 55 | 16.4 | 0.102 | 0.018 | 0.061–0.123 | + 0.4 | −12.5, +15.4 |
| 0.45–0.49 | 18 | 56 | 16.5 | 0.077 | 0.017 | 0.053–0.115 | − 0.2 | −13.4, + 7.6 |
| 0.40–0.44 | 7 | 56 | 16.5 | 0.071 | 0.014 | 0.064–0.097 | + 0.03 | − 9.8, + 5.6 |
| 0.35–0.39 | 1 | 53 | 14.7 | 0.061 | — | — | − 4.6 | — |
| **Second metabolism periods** | | | | | | | | |
| 0.70–0.74 | 0 | — | — | — | — | — | — | — |
| 0.65–0.69 | 2 | 68 | 18.2 | 0.092 | — | 0.084–0.099 | − 0.7 | − 7.6, + 6.2 |
| 0.60–0.64 | 2 | 59 | 16.7 | 0.126 | — | 0.124–0.129 | − 4.0 | −11.3, + 3.2 |
| 0.55–0.59 | 7 | 52 | 16.2 | 0.105 | 0.017 | 0.077–0.132 | + 2.6 | − 5.7, +11.6 |
| 0.50–0.54 | 12 | 56 | 17.3 | 0.083 | 0.021 | 0.059–0.121 | + 3.6 | −12, + 9.0 |
| 0.45–0.49 | 16 | 61 | 17.3 | 0.077 | 0.016 | 0.045–0.111 | + 0.5 | −12.3, +11.5 |
| 0.40–0.44 | 1 | 65 | 16.7 | 0.059 | — | — | − 3.5 | — |
| 0.35–0.39 | 0 | — | — | — | — | — | — | — |
| **Double metabolism periods** | | | | | | | | |
| 0.70–0.74 | 0 | — | — | — | — | — | — | — |
| 0.65–0.69 | 1 | 64 | 18.3 | 0.096 | — | — | + 2.6 | — |
| 0.60–0.64 | 2 | 54 | 17.3 | 0.090 | — | 0.087–0.094 | + 1.8 | − 4.9, + 8.5 |
| 0.55–0.59 | 7 | 55 | 16.7 | 0.079 | 0.016 | 0.056–0.105 | − 1.6 | − 7.9, + 4.3 |
| 0.50–0.54 | 3 | 54 | 15.6 | 0.081 | — | 0.065–0.092 | − 5.3 | − 8.0, − 3.9 |
| 0.45–0.49 | 1 | 62 | 21.0 | 0.062 | — | — | +16.6 | — |
| 0.40–0.44 | 6 | 59 | 16.5 | 0.073 | — | 0.054–0.098 | − 0.7 | − 5.2, + 4.5 |
| 0.35–0.39 | 2 | 56 | 15.7 | 0.056 | — | 0.054–0.059 | − 2.2 | − 4.1, − 0.4 |

average retentions based on actual weight during first and second periods were 0.083 and 0.078 gm., respectively, and 0.077 gm. during first and second periods when estimated on the theoretical weights. The closer agreement at these lower levels may be due to the fact that the children were more nearly physically fit as shown by the very slight variation from the theoretical weights, or there may have been too little protein in the diet to make possible the high retentions obtained at the higher ingestion levels. The range of retentions, however, would indicate that more nitrogen might have been retained by certain of the children had it been needed. With all groups above the minimum, the upper limit of retention was much higher than the lowest, or the average of the group.

High nitrogen retentions may not in all cases be due to previous nitrogen starvation, but rather to a greater need. The child destined to be tall will need more than the child who is inherently short. Retentions at ingestion levels of 0.40 to 0.44 gm. per kilogram suggest that possibly less than the optimum amount had been ingested at least for certain of the children studied, since low retentions were fairly consistent in both first and second runs, whether based on actual or theoretical weights, and the upper limit of retention is lower than those of the higher ingestion levels.

It should be noted that the retentions based on actual and theoretical weights at the various ingestion levels do not in all cases pertain to the same children, owing to the fact that estimations of ingestions based on theoretical weights sometimes placed certain of the children in a group above or below that estimated on actual weights. Moreover, the range of retentions at each ingestion level differed to a greater or less degree. The weight averages of the children of the various groups suggest that the high retentions may be due to the fact that the children were underweight, as suggested by Wang in explanation of certain high retentions among her groups. Therefore, in order to determine if these variations in retentions were due to weight variations of the children studied or to some other factor or factors, the retentions of

a few of the children at various ingestion levels have been grouped according to their actual and theoretical weights together with the percentage of weight variation from their theoretical average (table 3).

These data indicate that weight alone is not concerned with these variations in retentions, since high retentions were found among children who were above average in weight as

TABLE 3

*Comparison of nitrogen retentions of individual children in relation to actual and theoretical weight at various nitrogen ingestions*

| NAME | DATE | WEIGHT | BASED ON ACTUAL WEIGHT | | BASED ON THEORETICAL WEIGHT | | PER CENT OVER + OR UNDER − THEORETICAL WEIGHT |
|---|---|---|---|---|---|---|---|
| | | | Ingestion per kilogram | Retention per kilogram | Ingestion per kilogram | Retention per kilogram | |
| | | *kg.* | *gm.* | *gm.* | *gm.* | *gm.* | |
| G.O. | 5/20/33 | 16.78 | 0.70 | 0.107 | 0.65 | 0.099 | — 7.6 |
| R.H. | 5/20/33 | 19.01 | 0.61 | 0.079 | 0.64 | 0.084 | + 6.2 |
| L.T. | 5/20/33 | 15.79 | 0.68 | 0.143 | 0.61 | 0.129 | —11.3 |
| F.W. | 7/17/32 | 14.43 | 0.54 | 0.114 | 0.55 | 0.116 | + 0.9 |
| D.P. | 10/23/32 | 15.4 | 0.52 | 0.060 | 0.54 | 0.063 | + 2.9 |
| R.H. | 7/17/32 | 17.4 | 0.50 | 0.102 | 0.54 | 0.110 | + 8.2 |
| G.O. | 3/18/32 | 16.25 | 0.49 | 0.087 | 0.48 | 0.086 | — 3.3 |
| B.V. | 10/ 3/32 | 18.3 | 0.47 | 0.061 | 0.50 | 0.065 | + 6.5 |
| J.C. | 11/12/32 | 19.0 | 0.47 | 0.062 | 0.50 | 0.066 | + 6.4 |
| C.P. | 1/16/33 | 18.0 | 0.45 | 0.084 | 0.46 | 0.086 | + 2.3 |
| D.B. | 10/12/32 | 17.0 | 0.44 | 0.067 | 0.45 | 0.070 | + 3.7 |
| D.P. | 10/ 3/32 | 15.1 | 0.44 | 0.062 | 0.45 | 0.064 | + 1.5 |
| R.H. | 4/29/33 | 18.51 | 0.45 | 0.088 | 0.48 | 0.094 | + 5.8 |

well as with those below average. For example, D.B. and D.P. were quite normal children and, although very slightly above the average weight, were well within the range of normality. At ingestions of 0.45 gm. per kilogram (theoretical weight) they retained 0.070 and 0.064 gm. per kilogram, respectively; whereas C.P., at approximately the same ingestion, retained 0.086 gm. per kilogram, and R.H., at a very slightly higher ingestion (0.48 gm. per kilogram of theoretical

weight) retained 0.094 gm. per kilogram. During double metabolism periods (table 2), similar results were obtained; that is, high retentions were not in all cases correlated with underweight.

Objection may be made to the effect that the variation in the nitrogen retentions may be due in part to the kind of protein fed, possibly a larger proportion of high grade protein

TABLE 4

*Nitrogen retentions of children receiving protein from different sources*

| NAME | DATE | WEIGHT | PER CENT OVER + OR UNDER − THEORETICAL WEIGHT | NITROGEN INTAKE PER KILOGRAM | PROTEIN ANIMAL | NITROGEN EXCRETION | | NITROGEN RETENTION PER KILOGRAM |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Urine | Feces | |
| | | *kg.* | | *gm.* | *per cent* | *gm.* | *gm.* | *gm.* |
| R.H. | 5/12/33 | 18.8 | + 5 | 0.607 | 77 | 7.465 | 2.016 | 0.102 |
| L.T. | 5/12/33 | 16.65 | —11.3 | 0.660 | 52 | 7.164 | 1.534 | 0.111 |
| C.P. | 6/11/33 | 17.5 | — 4.8 | 0.597 | 62 | 7.527 | 1.192 | 0.098 |
| R.H. | 5/20/33 | 19.01 | + 6.2 | 0.606 | 77 | 8.357 | 1.662 | 0.083 |
| L.T. | 3/18/32 | 14 | — 5.4 | 0.58 | 82 | 6.260 | 0.749 | 0.081 |
| D.P. | 10/23/32 | 15.4 | + 2.9 | 0.52 | 81 | 5.816 | 1.264 | 0.060 |
| B.Mc. | 7/17/32 | 19.12 | + 5.9 | 0.52 | 72 | 6.795 | 1.451 | 0.092 |
| G.O. | 4/29/33 | 16.74 | — 7.1 | 0.51 | 49 | 5.570 | 1.507 | 0.091 |
| B.V. | 10/23/32 | 18.7 | + 6.9 | 0.49 | 75 | 6.588 | 1.319 | 0.070 |
| A.C. | 2/11/33 | 18.3 | +11.6 | 0.494 | 71 | 5.529 | 1.335 | 0.117 |
| E.E. | 2/ 3/33 | 22.6 | + 7.6 | 0.43 | 65 | 7.071 | 1.204 | 0.067 |
| J.C. | 11/ 4/32 | 18 | + 0.8 | 0.43 | 50 | 5.118 | 1.257 | 0.076 |
| C.P. | 5/ 1/32 | 17 | + 5.3 | 0.42 | 62 | 5.092 | 1.029 | 0.061 |
| D.S. | 11/ 4/32 | 17.1 | ± 0 | 0.425 | 53 | 5.082 | 1.379 | 0.047 |
| P.H. | 2/20/34 | 18.74 | + 8.5 | 0.576 | 77 | 8.021 | 1.251 | 0.079 |
| P.D. | 3/13/34 | 15.8 | — 0.6 | 0.415 | 58 | 4.323 | 1.033 | 0.077 |

being given in some cases than in others. The data, therefore, have been considered in relation to the per cent of animal protein (from 49 per cent to 82 per cent) in the various diets. Since it is not feasible to report the data of all the balance studies individually, a few typical records are given (table 4). From these it will be seen that, although the amount of animal protein of the diets differed, this apparently was not the influencing factor in the retention variations. P.D. and P.H.

retained very similar amounts of protein, 0.077 and 0.079 gm. per kilogram with ingestions of 0.415 and 0.576 gm. per kilogram, respectively. The lower protein diet contained 58 per cent of animal protein while the higher contained 77 per cent.

### *The relation of nitrogen retention to creatinine elimination*

The wide variations in the nitrogen retentions of the children studied even at the same ingestion levels suggest that studies of nitrogen needs based on weight alone may not determine the amount a given child should receive. A thin child may be very well developed and have little padding, while a well-padded child may have less than the normal amount of muscle development. The nitrogen retentions, therefore, have been studied in relation to muscle needs, estimating both ingestion and retention on the basis of the urinary creatinine.

The creatinine determinations by the Folin method were made in duplicate on each of the 24-hour urinary excretions which were collected under toluene and kept on ice, the average of the 5- or 10-day determinations being used. During certain periods, the diets included small amounts of meat given once a day. Tests with meat-free diets indicated that the errors introduced thereby when spread over the 24-hour period were not sufficient to invalidate the results. The nitrogen retention data per milligram of creatinine are grouped for the first and second metabolism periods on the basis of the ingestion per milligram at intervals of five with the corresponding nitrogen ingestions per kilogram of theoretical body weight of the children studied. Double metabolism periods are grouped with the second metabolism periods.

Nitrogen retentions studied from the standpoint of creatinine elimination indicate that at the higher level of ingestion more nitrogen was retained. Retentions during first metabolism periods also were higher than during second subsequent periods, suggesting that some of the children studied had been receiving previously less than the optimum amount of protein.

For example, at ingestion levels between 25 and 29 mg. of nitrogen per milligram of urinary creatinine, 5.1 mg. of nitrogen were retained during first periods and 4.6 mg. during second periods (table 5). At ingestion levels of 35 to 39 mg. of nitrogen per milligram of creatinine, 6.5 mg. and 5.9 mg. were retained during first and second metabolism periods, respectively. At the higher ingestions, similar results were

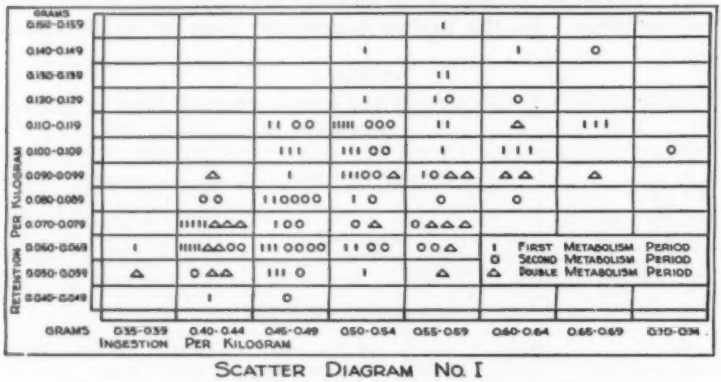
TABLE 5

*Relation between nitrogen ingestion and nitrogen retention based on creatinine excretion*

| NITROGEN INGESTION PER MILLIGRAM CREATININE | NUMBER OF CASES | AVERAGE AGE | NITROGEN RETENTION | | | OVER + OR UNDER − THEORETICAL WEIGHT | NITROGEN INGESTION | |
|---|---|---|---|---|---|---|---|---|
| | | | Average per milligram creatinine | Standard deviation | Range per milligram creatinine | | Average per kilogram theoretical weight | Standard deviation |
| **First metabolism periods** | | | | | | | | |
| *mg.* | | *mo.* | *mg.* | *mg.* | *mg.* | *per cent* | *gm.* | *gm.* |
| 45–49 | 3 | 37 | 11.2 | — | 9.1–13.0 | + 9.7 | 0.56 | — |
| 40–44 | 2 | 40 | 8.6 | — | 8.1– 9.0 | — 2.8 | 0.54 | — |
| 35–39 | 15 | 56 | 6.5 | 1.6 | 4.1–10.0 | — 1.0 | 0.56 | 0.05 |
| 30–34 | 21 | 57 | 5.7 | 1.2 | 3.9– 7.5 | + 0.5 | 0.49 | 0.04 |
| 25–29 | 15 | 63 | 5.1 | 0.9 | 2.9– 6.4 | + 1.6 | 0.47 | 0.05 |
| 20–24 | 3 | 63 | 3.8 | — | 3.3– 4.6 | + 2.9 | 0.43 | — |
| **Second metabolism periods** | | | | | | | | |
| 45–49 | 2 | 38 | 9.2 | — | 8.7– 9.7 | + 1.5 | 0.55 | — |
| 40–44 | 1 | 54 | 6.3 | — | ——— | — 4.9 | 0.61 | — |
| 35–39 | 18 | 53 | 5.9 | 1.4 | 3.8– 8.6 | — 0.1 | 0.57 | 0.04 |
| 30–34 | 20 | 58 | 5.0 | 1.0 | 3.5– 8.0 | + 0.0 | 0.51 | 0.05 |
| 25–29 | 16 | 62 | 4.6 | 0.8 | 2.8– 6.0 | + 1.2 | 0.46 | 0.05 |
| 20–24 | 4 | 65 | 3.8 | — | 2.8– 4.6 | + 7.0 | 0.44 | — |

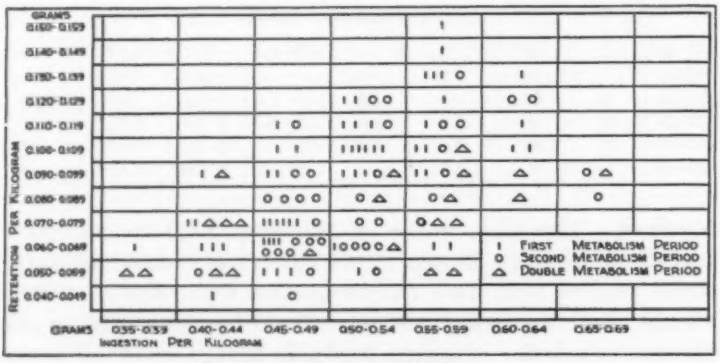
obtained. The significance of the very high retentions at ingestions of 45 to 49 mg. is not clear. The data pertain to three studies with two children, who were above average weight, but somewhat younger than the majority of the children studied. It seems probable that a child of 3 may need more protein per unit of muscle mass than a child of 6. The data based on weights, however, suggest that the variation in age of the children studied was not a significant factor influencing the retentions (table 1).

The range of nitrogen retentions based on the creatinine eliminations (table 5; scatter diagram 3) during the second metabolism periods would seem to indicate that ingestions between 30 and 34 mg. per milligram will meet the needs of the average child, since the highest retention of the group,




SCATTER DIAGRAM NO. I

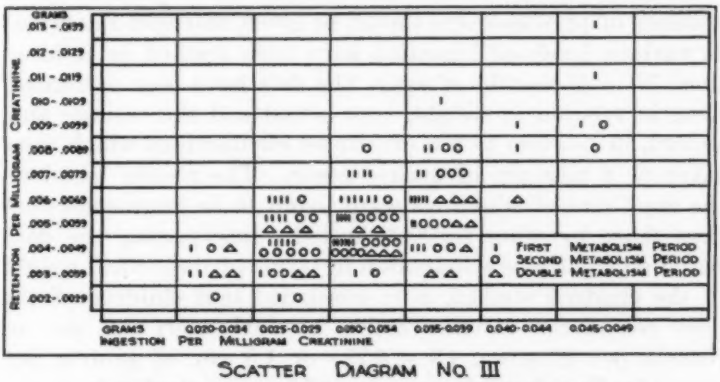
SHOWING THE RELATION OF NITROGEN INGESTION TO NITROGEN RETENTION BASED ON THE ACTUAL WEIGHTS OF THE CHILDREN STUDIED




SCATTER DIAGRAM NO. II

SHOWING THE RELATION OF NITROGEN INGESTION TO NITROGEN RETENTION BASED ON THE THEORETICAL WEIGHTS OF THE CHILDREN STUDIED

8 mg. per milligram of creatinine is approximately the same as that of the highest retention—8.6 mg. per milligram—of the next higher ingestion level, 35 to 39 mg. Nitrogen ingestions of 30 to 34 mg. per milligram of creatinine considered in relation to the theoretical weights of the children studied were found to be equivalent to 0.51 gm. of nitrogen per kilogram, or 3.2 gm. of protein per kilogram—an amount not far from that reported by Holt in the study of the protein taken by children of similar ages, nor from the ingestions




SCATTER DIAGRAM NO. III
SHOWING THE RELATION OF NITROGEN INGESTION TO NITROGEN RETENTION BASED ON THE URINARY CREATININE ELIMINATION OF THE CHILDREN STUDIED

(0.24 gm. of nitrogen per pound) with which Wang, Hawks and Kaucher ('28) obtained their highest retentions in children of normal weight.

Nitrogen retentions studied from the standpoint of either body weight or muscle development seem to point to the conclusion that children of the preschool age need more protein than we have hitherto appreciated. Instead of 1 gm. per pound, the amount usually considered adequate, approximately 1.5 gm. per pound would seem to be more nearly the requirement of the majority of children of preschool age. The lower levels used in the study as well as those reported

by Parsons and Bartlett were sufficient to cover katabolic processes since no child in any of the groups was found to be in negative balance. At these lower levels, furthermore, the children were able to gain in weight and increase in stature. Whether children receiving these lower amounts are less physically fit than those who receive higher levels of ingestion, resulting in higher retentions, remains to be determined.

## SUMMARY

In an endeavor to determine the amount of protein which children of preschool age should be given, nitrogen retentions at various levels of ingestion have been studied in children from 37 to 66 months of age. The data have been evaluated, first, in relation to weight, both actual and theoretical; and, second, in relation to the creatinine eliminations which were taken as a measure of muscle needs. The results based on the creatinine eliminations were found to be more consistent than those by other methods tested. Interpreting the data based on creatinine elimination in terms of theoretical weight of the children studied, it is concluded that children of the ages considered should receive approximately 3.2 gm. of protein per kilogram (N.$\times$ 6.25) or 1.5 gm. of protein per pound in diets furnishing at least 50 per cent of the protein from animal sources.

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# A STUDY OF THE BASAL METABOLISM AND DIET OF NORMAL YOUNG COLLEGE WOMEN IN FLORIDA

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Observations made by Hafkesbring and Borgstrom ('26), Tilt ('30), Remington and Culp ('31), Coons ('31) and Coons and Schiefelbusch ('32) show that the basal metabolic rates of women living in various places in the southern parts of the United States are somewhat lower than the rates of those living in northern parts. The suggestions have been made that a low protein and calorie intake as well as climate might be factors influencing basal metabolism. This study has been made on a group of thirty normal young women, 17 to 26 years of age, for the purpose of adding to the existing data on basal metabolism observations of young women in warm climates. In addition, the average daily protein and calorie intake of eighteen of the group was determined in order to study a possible relationship between basal metabolism and protein and calorie intake.

## EXPERIMENTAL

The subjects selected for this study were young women students at the Florida State College for Women, ranging from 17 to 26 years of age. Seventeen of the young women were native Floridians, while thirteen had lived in Florida from 8 to 23 years. Eight of the subjects had recently come from northern states. All subjects were considered normal individuals as shown by health records and examinations by

the college physician. Basal metabolism observations were made on the northern group and on thirteen of the Florida group once during the year. Observations were made on the remaining eighteen during the fall, winter, and spring. A 4-day dietary study was made on the group of eighteen in order to determine whether a relationship might exist between diet and basal metabolism.

*Basal metabolism.* The subjects came to the laboratory early in the morning, 12 to 14 hours after the last meal. After a 40- to 60-minute rest period on comfortable beds, the basal metabolism was observed. A Benedict-Roth apparatus with kymograph attachment was used. Two 8- to 10-minute observations were made during one test period. Four or more observations were made on each subject. The average of those observations agreeing within 5 per cent was considered the basal metabolic rate. Observations during the menstrual period were not avoided unless discomfort was experienced.

Every effort was made to secure accurate determinations. The apparatus was checked for leaks by placing two 20-gm. weights on the spirometer bell for 2 or more minutes during each test. After each morning's observations the residual gas in the spirometer was passed through barium hydroxide or lime water to check for complete carbon dioxide absorption. Since it was impossible to make an alcohol check on the apparatus, two reliable subjects were observed with a Sanborn graphic apparatus in the college infirmary and a Benedict-Roth apparatus at a doctor's office in Tallahassee. The very close checks obtained would indicate that the apparatus was accurate.

The calories per hour were determined and deviations calculated from the following prediction standards: Aub-DuBois, as modified by Boothby and Sandiford ('29); Harris-Benedict, which was extrapolated for those under 21 years of age; and the standard derived from Benedict's Girl Scout studies, making a 10 per cent correction for subjects awake, for those 17 and 18 years old.[1]

[1] Personal communication.

*Dietary study.* A study of the protein and calorie intake was made over a period of 4 days, during which time the young women followed their regular dietary habits. All meals were eaten in the college dining hall. The food served at each meal and that eaten between meals was collected and prepared for analysis by the method of Benedict and Farr ('29). The protein constant was determined by the Kjeldahl method (Bodansky, '28); the calorie value by the oxy-calorimeter method (Benedict and Fox, '25). From these data the protein and calorie intake of each subject was calculated.

## RESULTS AND DISCUSSION

*Basal metabolism.* The calories per hour observed on eighteen subjects for different seasons of the year are given in table 1. There appears to be no consistent variation.

TABLE 1

*Variation in heat production during fall, winter, and spring*

| SUBJECT NO. | CALORIES PER HOUR, OBSERVED | | | |
|---|---|---|---|---|
| | October–November | February | April | Average |
| 2 | 50.59 | 53.52 | 51.56 | 51.89 |
| 6 | 46.47 | 46.70 | 47.90 | 47.02 |
| 7 | 50.83 | 54.23 | 52.92 | 52.66 |
| 9 | 44.15 | 42.45 | 43.56 | 43.39 |
| 11 | 52.07 | 50.34 | 52.95 | 51.79 |
| 12 | 50.63 | 49.72 | 48.72 | 49.69 |
| 13 | 49.37 | 53.61 | 51.13 | 51.37 |
| 15 | 50.66 | 49.25 | 51.47 | 50.46 |
| 17 | 54.07 | 54.10 | 53.49 | 53.89 |
| 19 | 47.69 | 46.31 | 46.49 | 46.83 |
| 20 | 48.11 | 46.57 | 46.95 | 47.21 |
| 21 | 48.89 | 47.99 | 48.52 | 48.47 |
| 22 | 48.33 | 50.22 | 57.72 | 52.09 |
| 23 | 57.37 | 59.61 | 59.38 | 58.79 |
| 25 | 44.43 | 44.93 | 44.45 | 44.60 |
| 28 | 53.05 | 53.76 | 54.15 | 53.65 |
| 29 | 51.13 | 51.06 | 49.80 | 50.66 |
| 30 | 48.60 | 49.79 | 48.49 | 48.96 |

Subjects nos. 2, 7, and 13 show an increase of 5.7 per cent, 6.7 per cent, and 8.6 per cent, respectively, in the basal metabolism observed in February over that observed in October or November. One subject, no. 22, shows an increase of 14.9 per cent in the calories per hour observed in April over the observation in February. It would appear, therefore, that in this climate where temperature changes are not great there is little, if any, seasonal variation. This is in accord with former observations in this laboratory (Tilt, '30). The

TABLE 2

*Basal metabolism of normal young women living in Florida. Averages according to age group*

| NUMBER IN GROUP | AGE | | HEIGHT | WEIGHT | SURFACE AREA | CALORIES PER HOUR OBSERVED | DEVIATION OF OBSERVED FROM PREDICTION | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Aub-DuBois modified prediction | Harris-Benedict prediction | Benedict girl scout prediction |
| | *yr.* | *mo.* | *cm.* | *kg.* | *sq.m.* | | *per cent* | *per cent* | *per cent* |
| 1 | 17 | 5 | 161.0 | 52.8 | 1.53 | 54.92 | − 4.0 | − 3.6 | − 4.0 |
| 6 | 18 | 5 | 165.3 | 56.1 | 1.61 | 51.29 | − 14.5 | − 13.0 | − 10.1 |
| 6 | 19 | 4 | 165.6 | 58.1 | 1.63 | 49.75 | − 17.6 | − 16.4 | |
| 7 | 20 | 6 | 163.1 | 55.6 | 1.59 | 49.94 | − 14.3 | − 14.0 | |
| 5 | 21 | 4 | 165.3 | 57.3 | 1.61 | 51.73 | − 13.4 | − 12.1 | |
| 2 | 23 | 6 | 163.1 | 60.8 | 1.64 | 53.45 | − 11.7 | − 10.1 | |
| 2 | 24 | 2 | 165.2 | 55.5 | 1.59 | 52.16 | − 11.4 | − 9.2 | |
| 1 | 25 | 8 | 161.0 | 55.0 | 1.56 | 48.96 | − 13.2 | − 13.4 | |
| Average | | | | | | | − 14.1 | − 13.0 | − 9.2 |

few variations which do occur cannot be accounted for, since the young women seemed to have made no noticeable changes in their habits.

Since there appeared to be no significant seasonal variation, the calories per hour observed for each season were averaged to obtain the average basal metabolism of these eighteen young women. The results of these determinations, averaged, and those on the additional twelve upon whom observations were made during only one season, all grouped according to age, are given in table 2.

The average deviation of the group is –14.1 per cent from the prediction of Aub-DuBois, modified by Boothby and Sandiford, and –13.0 per cent from the Harris-Benedict standard. There is considerable individual variation from these averages. Eighty per cent of the entire group are below the –10 per cent limit of the Aub-DuBois prediction and 76 per cent from the Harris-Benedict. Fifty per cent (Aub-DuBois) and 43 per cent (Harris-Benedict) are below the –15 per cent limit. Only six of the thirty studied were above –10 per cent (Aub-DuBois), the highest being – 2.2 per cent; three were below – 20 per cent, the lowest – 24.4 per cent. According to the Harris-Benedict standard seven were above –10 per cent, the highest was – 0.2 per cent; three were below – 20 per cent, the lowest observed being – 21.6 per cent. The subject who had the highest metabolism had lived in Florida 10 years, but spends the summers in Indiana.

These results are lower than those previously reported for Florida women of the same ages (Tilt, '30), when an average of –10.6 per cent (Aub-DuBois) and – 9.9 per cent (Harris-Benedict) were found. Although the average is slightly below the –13.5 per cent (Aub-DuBois) reported by Coons ('31) for Oklahoma women of the same age groups, the percentages falling below the –10 and –15 per cent limits are somewhat higher. However, Coons observed lower rates, one – 28.6 per cent (Aub-DuBois) and two cases – 27 per cent. Remington and Culp ('31) reported an average of –10.4 per cent (Aub-DuBois) for a group of South Carolina women.

The results of basal metabolism observations on the group of eight normal young women who had recently come from northern states are given in table 3. It is interesting to note that the average percentage deviation from the Harris-Benedict standard (extrapolated) is – 7.8 per cent, a figure very near the –7.1 per cent obtained by Hetler ('32) and the – 7.6 per cent by Stark ('33) for young women of approximately the same age. The range within the group is from –12.3 per cent to – 2.8 per cent (Aub-DuBois, modified), and –13.5 per cent to –1.7 per cent (Harris-Benedict).

*Dietary study.* From a study of table 4 which summarizes the average daily intake of each individual, it is noted that most of the diets were quite adequate from the standpoint of grams protein and total calories. The average protein intake is 1.23 gm. per kilogram per day. This value is considerably higher than the 0.94 gm. per kilogram observed by Hetler ('32). It is also somewhat higher than the figures reported for medical students at New Orleans (Denis and Borgstrom, '24), Cleveland (Beard, '27), and Chapel Hill (Brooks, '29), which average slightly above 1 gm. per kilogram. Only three subjects (nos. 7, 11, and 20) ate less than the Sherman ('33) standard of 1 gm. per kilogram of body weight. The quality of the protein consumed appears to be quite adequate for meat, milk, and eggs were well distributed throughout the diets. The calorie intake was calculated on the average daily intake per kilogram of body weight. The average is 39.9 calories. This would be considered sufficient for the activity of these young women. The calorie intake is low for the same subjects whose protein intake is low.

TABLE 3

*Basal metabolism of normal young women from northern states. Average according to age groups*

| NUMBER IN GROUP | MONTHS IN FLORIDA | AGE | | HEIGHT | WEIGHT | SURFACE AREA | CALORIES PER HOUR OBSERVED | DEVIATION OF OBSERVED FROM PREDICTION | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Aub-DuBois modified prediction | Harris-Benedict prediction | Benedict girl scout prediction |
| | *mo.* | *yr.* | *mo.* | *cm.* | *kg.* | *sq.m.* | | *per cent* | *per cent* | *per cent* |
| 4 | 4 | 17 | 3 | 158.6 | 51.8 | 1.50 | 51.22 | − 8.7 | −9.9 | −10.6 |
| 2 | 10 | 18 | 6 | 164.1 | 56.6 | 1.61 | 53.64 | −10.1 | −8.9 | − 7.2 |
| 1 | 6 | 19 | 5 | 166.0 | 52.1 | 1.57 | 56.10 | − 3.9 | −1.7 | |
| 1 | 5 | 21 | 4 | 158.2 | 54.4 | 1.53 | 54.84 | − 2.8 | −3.2 | |
| Average | | | | | | | | − 7.7 | −7.8 | − 9.5 |

*Basal metabolism and protein and calorie intake.* There does not appear to be any definite parallelism between protein and calorie intake and basal metabolism. Subject no. 23,

who shows a basal metabolism of – 2.4 per cent (Aub-DuBois) and – 0.2 per cent (Harris-Benedict), which is the highest of the group, had an average protein intake of 1.64 gm. per kilogram of body weight per day. This is considerably more than that of others in the group. The daily calorie intake of

TABLE 4

*Average protein and calorie intake and the basal metabolism of a group of young women living in Florida*

| SUBJECT NO. | PROTEIN INTAKE | | CALORIE INTAKE | | AVERAGE BASAL METABOLIC RATE | |
|---|---|---|---|---|---|---|
| | Average per day | Average per kilogram body weight per day | Average per day | Average per kilogram body weight per day | Aub-DuBois prediction | Harris-Benedict prediction |
| | *gm.* | *gm.* | | | *per cent* | *per cent* |
| 2 | 69.21 | 1.10 | 2268 | 42.1 | – 12.1 | – 11.4 |
| 6 | 60.49 | 1.18 | 1981 | 39.2 | – 16.9 | – 16.7 |
| 7 | 49.97 | 0.82 | 1662 | 27.3 | – 13.7 | – 12.8 |
| 9 | 57.45 | 1.27 | 1886 | 41.5 | – 18.5 | – 20.0 |
| 11 | 67.70 | 0.96 | 2090 | 29.8 | – 24.4 | – 21.3 |
| 12 | 56.23 | 1.09 | 1960 | 37.9 | – 13.1 | – 12.4 |
| 13 | 72.53 | 1.25 | 2241 | 38.6 | – 15.1 | – 13.7 |
| 15 | 76.75 | 1.41 | 2424 | 45.7 | – 11.9 | – 11.9 |
| 17 | 77.30 | 1.48 | 2520 | 42.7 | – 13.0 | – 10.5 |
| 19 | 65.91 | 1.21 | 2356 | 43.2 | – 18.6 | – 18.1 |
| 20 | 47.77 | 0.90 | 1451 | 27.3 | – 16.5 | – 16.7 |
| 21 | 62.46 | 1.38 | 2184 | 39.7 | – 18.4 | – 16.5 |
| 22 | 69.72 | 1.08 | 2239 | 34.7 | – 17.6 | – 15.4 |
| 23 | 90.94 | 1.64 | 2869 | 51.6 | – 2.4 | – 0.2 |
| 25 | 60.79 | 1.12 | 2148 | 39.5 | – 20.0 | – 21.6 |
| 28 | 77.78 | 1.41 | 2409 | 43.5 | – 9.7 | – 7.2 |
| 29 | 67.90 | 1.26 | 2234 | 40.7 | – 13.0 | – 11.2 |
| 30 | 80.17 | 1.49 | 2720 | 53.7 | – 13.2 | – 13.4 |
| Average | 67.23 | 1.23 | 2202 | 39.9 | – 14.9 | – 13.9 |

this subject was also high, averaging 51.6 calories per kilogram of body weight. In the case of subject no. 11, who had the lowest basal metabolism (Aub-DuBois, – 24.4 per cent; Harris-Benedict, – 21.3 per cent), there was a daily intake of 0.96 gm. protein and 29.8 calories per kilogram of body weight. Although the basal metabolism observations were not coincident with the dietary study, it is believed that the diet for the

4-day period in October was representative of the customary diet while in college, at least. The fact that all of the young women eat in the college dining hall where a carefully planned diet of approximately the same food value throughout the year is served may account for the differences observed by Coons and Schiefelbusch ('32). Their study showed the calorie intake to be barely adequate, and the protein, although adequate in quantity, appeared to be deficient in quality.

## SUMMARY

1. Basal metabolic rates were determined on a group of thirty normal young women who have lived in Florida 8 years or longer. The average deviations calculated from prediction standards were: Aub-DuBois, modified by Boothby and Sandiford, –14.1 per cent; Harris-Benedict, –13.0 per cent.

2. Basal metabolic rates were determined on a group of 8 young women who had recently come from northern states. The average deviations were – 7.7 per cent from the modified Aub-DuBois standard, – 7.8 per cent from the Harris-Benedict (extrapolated).

3. A comparison of the average basal metabolism of young women living in Florida with those from northern states shows a decidedly lower rate for Florida women.

4. A 4-day dietary study on eighteen young women of the group living in Florida showed an average intake of 1.23 gm. protein and 39.9 calories per kilogram of body weight per day. The protein was of good quality.

5. No consistent relationship was noted between basal metabolism and protein or calorie intake.

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